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about to eat a
Leopard frog (*Rana pipiens*)
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The ins

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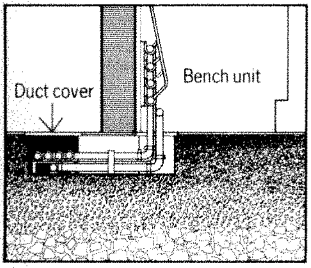

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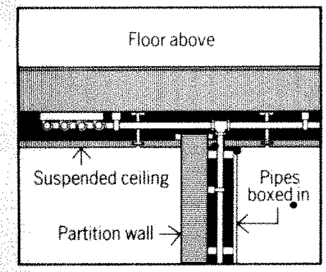

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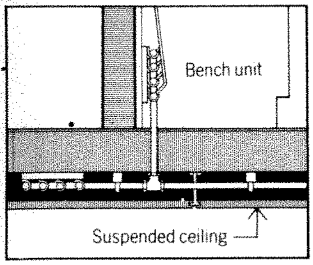

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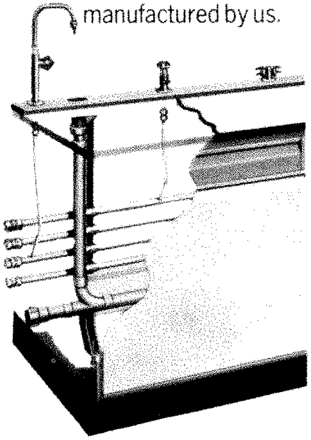

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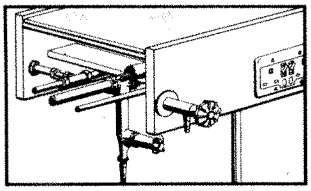

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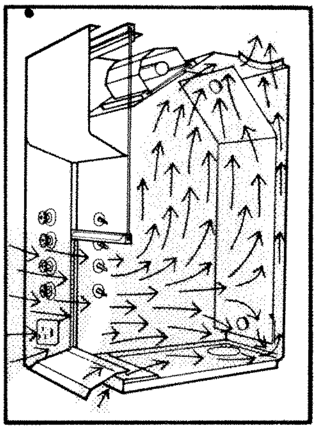

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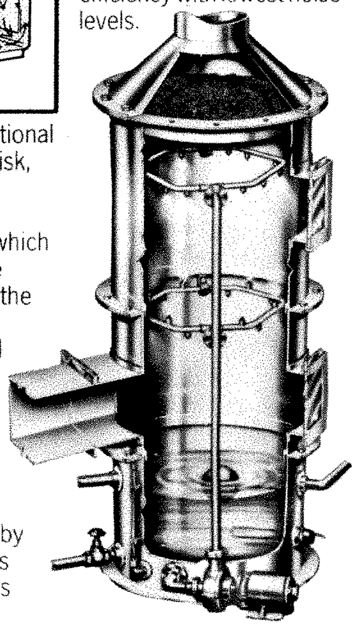

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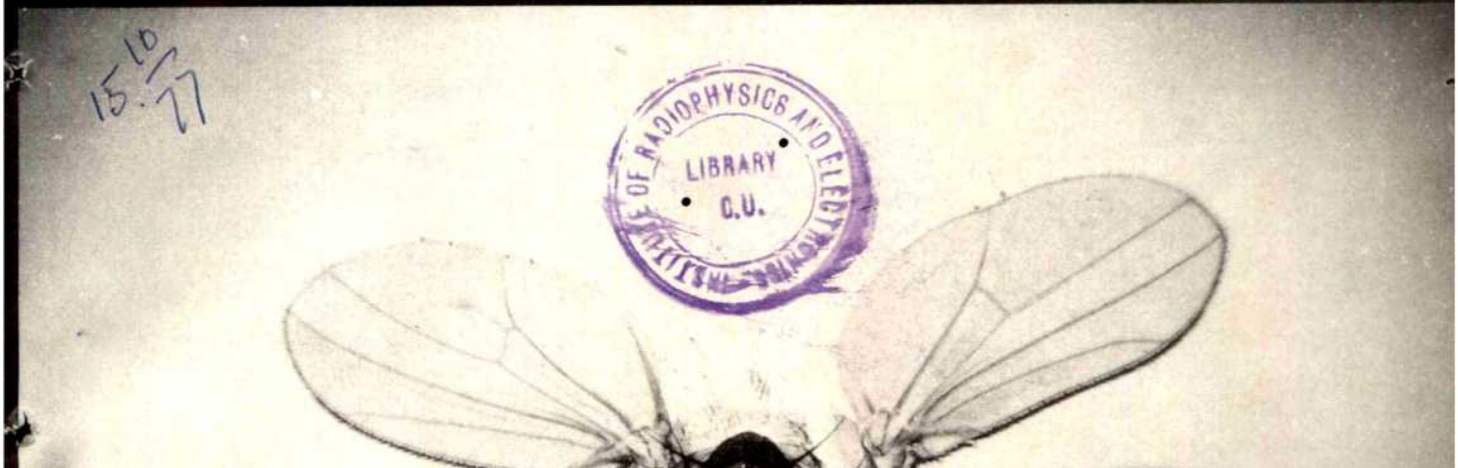

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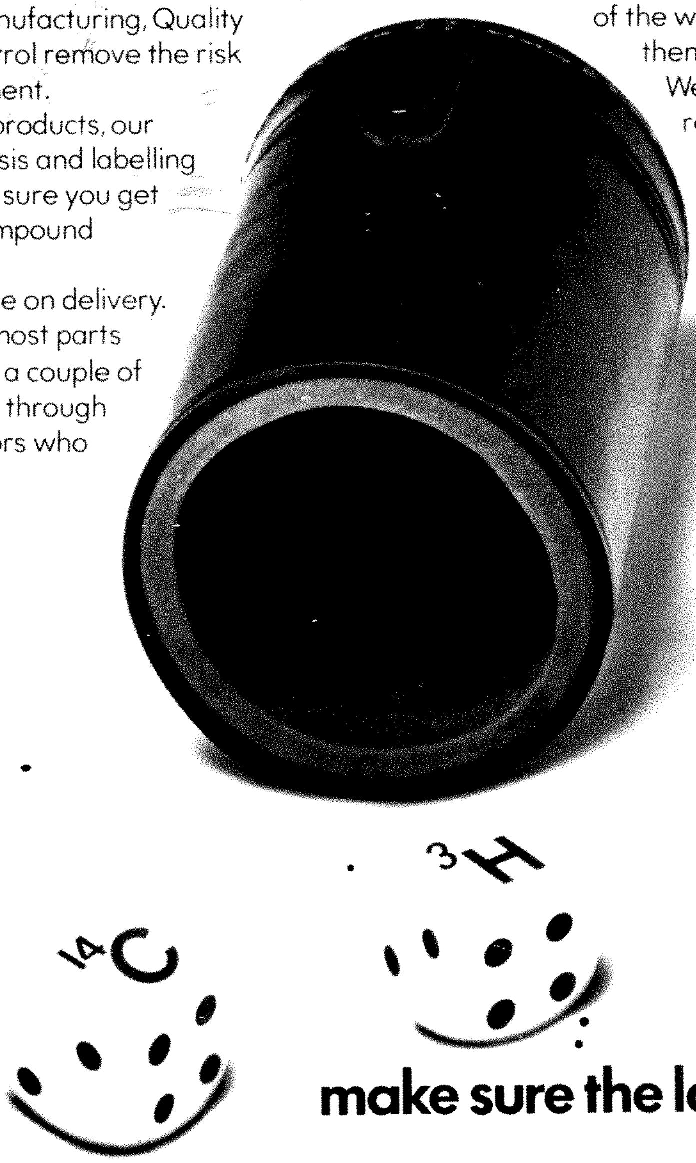

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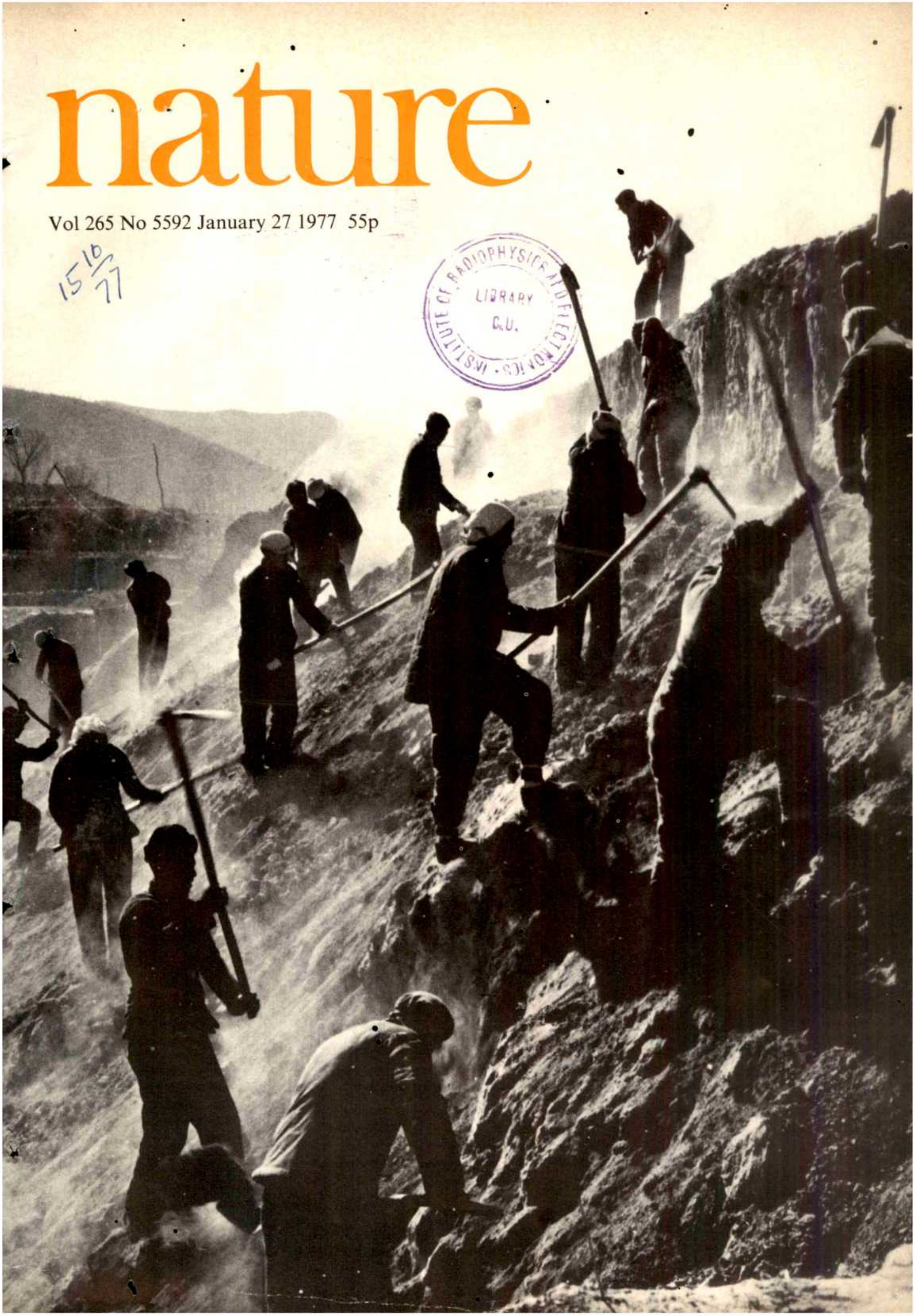

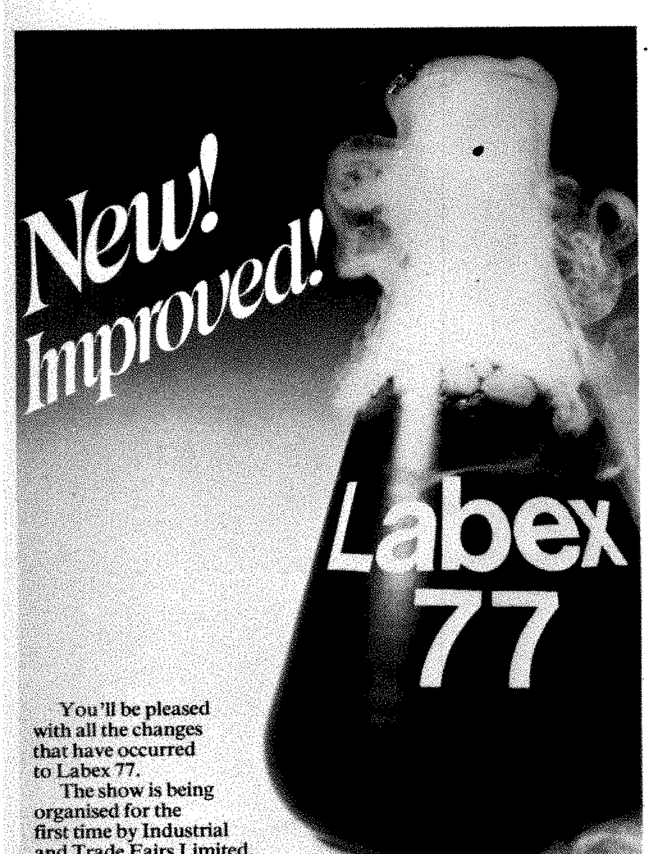

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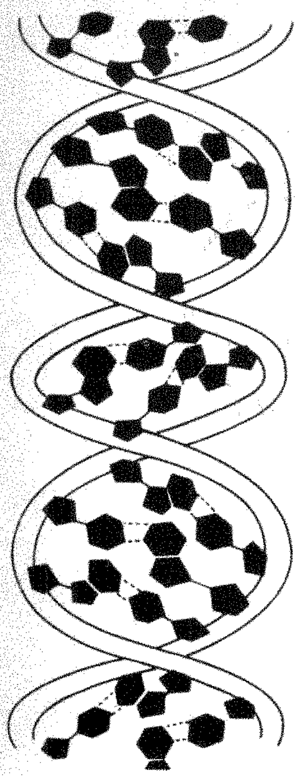

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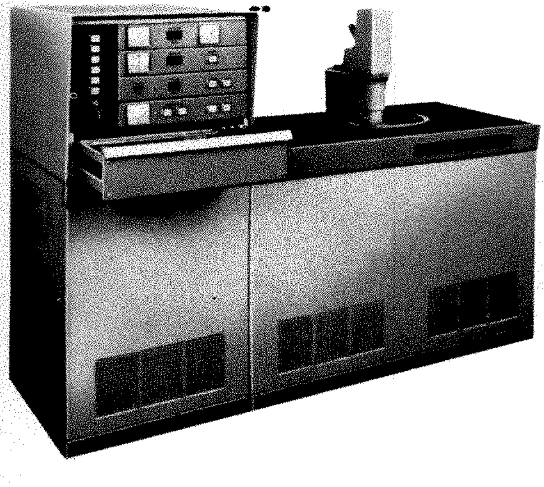

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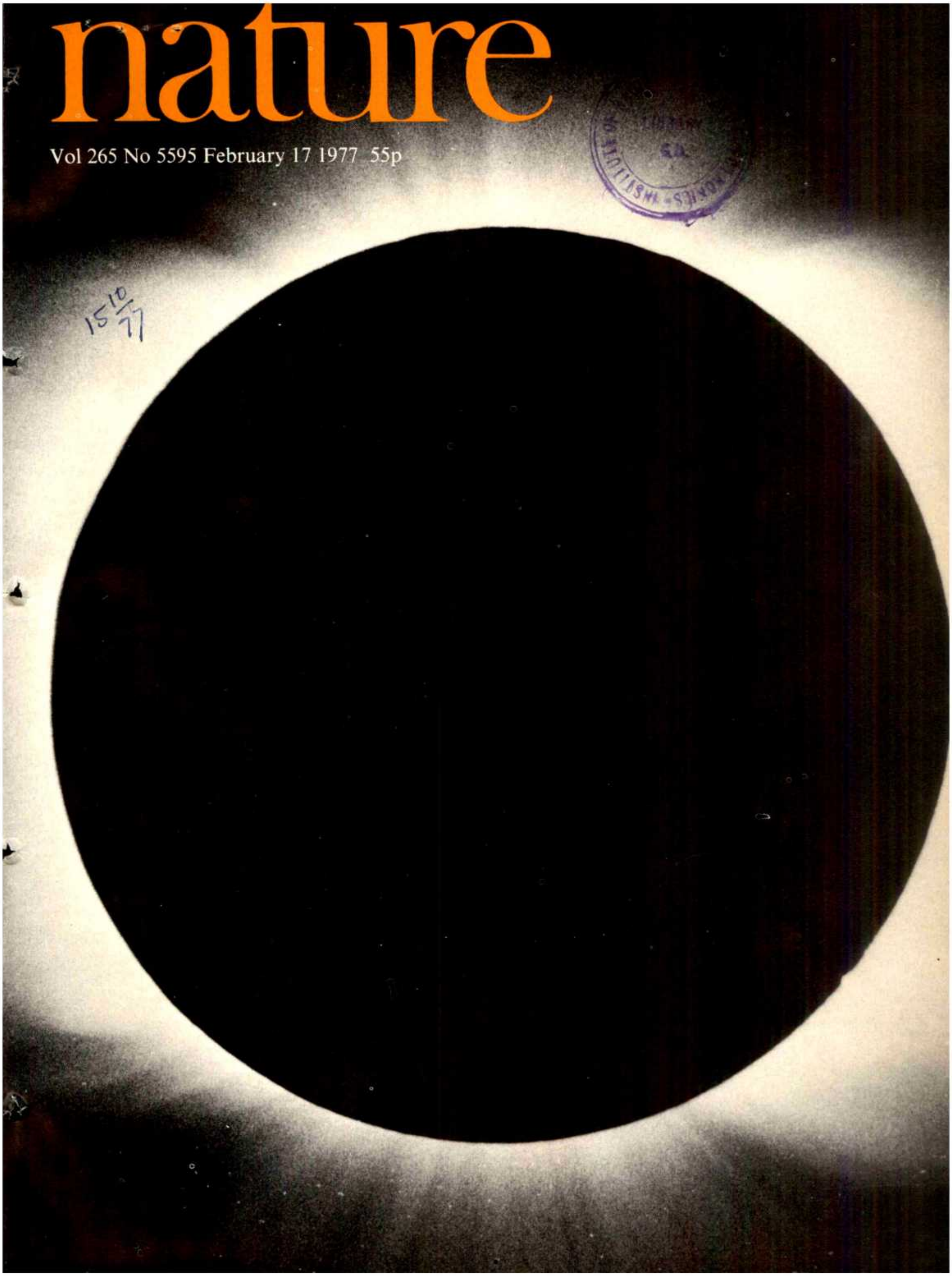

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Total solar eclipse, October 23, 1976. Photograph taken in Zanzibar by Dr R. C. Maddison, University of Keele. On page 611, M. Waldmeier describes his observations of the corona during this eclipse.

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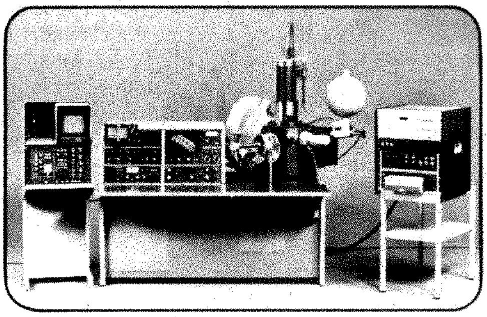

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- 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

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The French Institute of Health and Medical Research (Institut National de la Santé et de la Recherche Médicale) is organizing a series of meetings on advanced research topics in various biomedical fields. The meetings which will be held annually as of November 1977, will be known as the INSERM CONFERENCES.

The aims of the conferences are:

- **to foster the exchange of ideas, to evaluate new methods and new lines of investigation;**
- **to bring together scientists, mostly from European countries, working in universities and public or private research institutions.**

The meetings will be held under the direct responsibility of a chairman and a co-chairman who will be chosen each year with the agreement of the Conference participants.

The INSERM CONFERENCES will have the following special features:

- **only highly topical subjects will be dealt with;**
- **if possible, the data presented should not have been the subject of any earlier complete publication, a condition which therefore excludes general reviews;**
- **the Conferences will not publish proceedings or any other material, even in a summarized form;**
- **the chairman of each INSERM Conference will invite 15 to 20 scientists to give a report in line with the above conditions. The number of contributions will be limited to three or four per session and, at the discretion of the chairman, at least one third of the time will be given over to discussion and brief informal communications.**

In addition to the invited speakers, at least fifty participants will attend the meeting, and efforts will be made to select young scientists. Experienced research workers from fields other than those relating to the Conference will also be welcome to attend. Participants will be chosen in such a way as to enable those engaged in all types of scientific research to establish personal contacts, exchange information and find new ways of working together.

PRACTICAL ARRANGEMENTS

The INSERM CONFERENCES will be held at the Domaine de Seillac, near Blois, during the month of November 1977 (full address: Domaine de Seillac, 41150 Seillac, France - 180 km from Paris). Each conference will last three and a half days, from Sunday evening (departure from Paris), to Thursday afternoon. Working sessions will be held from 9 a.m. to 12.30 p.m. and from 5.30 p.m. to 8 p.m. On free afternoons, participants will have a wide choice of leisure activities at the Domaine de Seillac and in the surrounding area.

During May 1977, the final programme for each INSERM Conference, along with the registration form will be published in this journal. Those wishing to receive additional information as of now, should complete and forward the enclosed form.

REGISTRATION FEE AND SPECIAL FUND

Participants whose applications are accepted but who are not invited speakers, will be asked to pay their registration fee and board (1000 FF). A special fund will be made available to the chairman of each Conference, enabling him to pay part of the expenses of some participants requesting such assistance.

PROGRAMME FOR 1977

The first year of INSERM CONFERENCES will include three meetings as follows:

1977 ENDOCRINOLOGY

November 7 - 10

Chairman: Jacques HANOUNE
Co-Chairman: Etienne BAULIEU

Presentations will concern the following fields of interest:

1) Recent methodological advances and new biological systems;
2) Hormonal regulation of the intermediary metabolism;
3) Membranes and transduction of hormonal information.

1977 IMMUNOLOGY

November 14 - 17

Chairman: François KOURILSKY
Co-Chairman: J.-F. BACH

Presentations will concern the following fields of interest:

1) Nature and specificity of T-cell receptors - Relationships with the major histocompatibility complex;
2) T-cell mediated cytotoxicity.

1977 NEUROBIOLOGY

November 21 - 24

Chairman: Jacques GLOWINSKI
Co-Chairman: J.-P. CHANGEUX

Presentations will concern the following fields of interest:

1) Neurotransmitters - Identification; synthesis and release processes;
2) Receptors - Characterization; isolation; molecular properties and regulation.

Additional information about INSERM CONFERENCES, along with the registration forms and conference programmes, may be requested by completing the enclosed reply form and forwarding it before April 1st to **INSERM CONFERENCES, Institut National de la Santé et de la Recherche Médicale, 101, rue de Tolbiac - 75645 PARIS CEDEX 13, France** - Tél. : 584.14.41.

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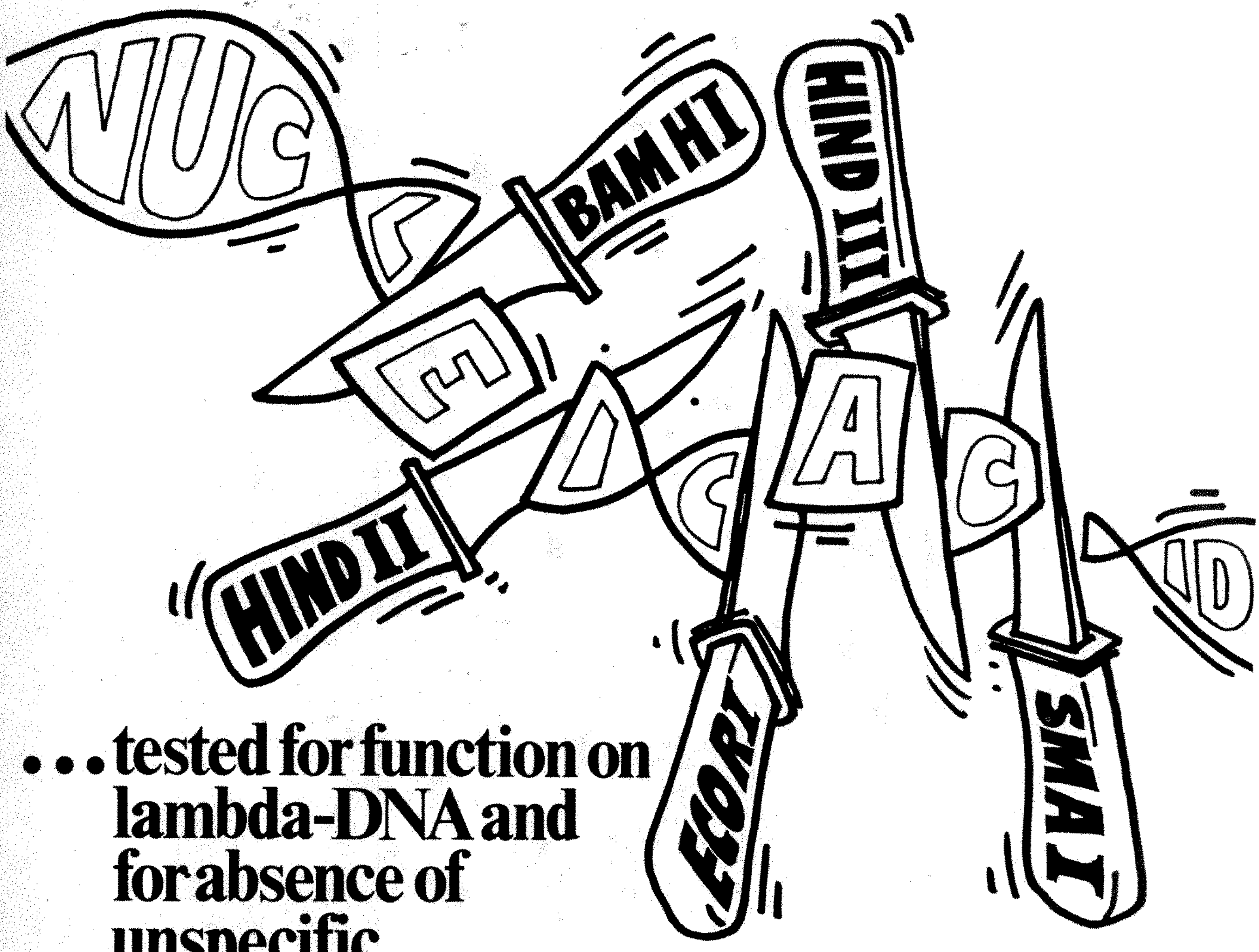

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Cover picture

Bacteriophage ΦX174 for the exposition of its complete genome.

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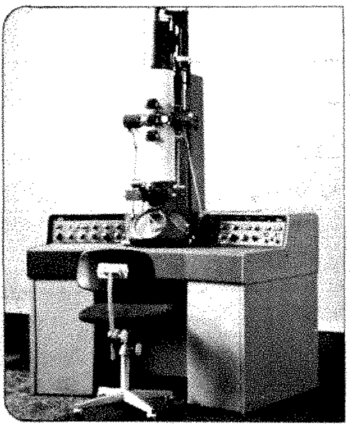

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January 6, 1977

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Back to the freeze?

A COUPLE of years ago (January 10, 1975) we reported on an extremely gloomy set of figures which showed how openings for senior university posts in the United Kingdom had all but disappeared in 1974 as a result of a general freeze in hiring. We have now brought the figures up to date by analysing advertisements for the years 1975 and 1976. Things are, at least temporarily, much better.

In the past two years the level of hiring seems to have been back fairly close to the levels of the late 1960s and early 1970s. Of course this indicates that the backlog has not been, and is never likely to be cleared. Some posts have disappeared for ever. But it may help to shake loose, in the course of time, the rigidity with which the universities were beginning to be afflicted. That is, unless the next rather ominous cloud on the horizon can be dispersed.

That cloud is the recent warning by the University Grants Committee that next year's (1977–8) grant to universities for salaries, maintenance and capital expenditure could be as much as 4% down in real terms. This figure first appeared in mid-1976 when the raising of student fees was seen by the Department of Education and Science not only as a way of bringing in more revenue to universities but also as a restraint on growth in student numbers and therefore in university financial needs. Since then there has been much re-adjustment, including the recent public expenditure cuts, but the result of it all is that we are back to the figure of 4% as the basis for discussion and warning.

Three quarters of all UGC disbursements go on salaries and so the next round of the national pay policy, not yet openly discussed, could make the figure look disastrous—or even quite tolerable; such is the uncertainty in which universities, once used to quinquennial planning, now live. But whatever happens there is a limit to the amount that can be saved by not painting, not cleaning and not heating. Very soon we could be back to solving the universities' financial problem by again venturing on the capricious course of not hiring. ☐

Amaze, test, shock your friends

THE festive season permits us to see ourselves as others see us and no better than through the scientific books, toys and kits showered on our children—and ourselves.

Here we have a book in which it says that if you drop a slice of buttered bread from a height it will land more often with its buttered side down. This is because the layer of butter makes one side heavier so "there is a tendency for the butter-side to fall faster". Yet further on we are told that in the absence of air "heavy things and light ones get up speed with the same degree of acceleration". And to complete the confusion we hear about a lorry with a load of canaries. When the birds all fly up in their cages "the weight on the tyres is reduced".

Or here is an excellent planisphere to show the position of the stars. The stars, it says on the back "appear to move in the same way as the sun" except that the "apparent movement of constellations near Polaris is more limited". And your absolute beginner, venturing out just after Christmas in the early evening might wonder why a couple of the most prominent 'stars' in the sky don't rate a mention.

They do get mentioned elsewhere in an encyclopedia for boys and girls. "The Universe is made up of the Earth we live on, the Sun and its other planets, thousands of other small bodies—and space itself . . . a planet is a big ball of rock and gas".

Nothing about astronomy in the Super Quiz Book for Girls; the question on stars runs "where were Paul Newman and Omar Sharif born?" In the same book there is a picture of a lady in a white coat which bears the label 'UKAEA'. A machine is applying lipstick by remote control—"Of course, the machine was developed for handling radioactive materials, but something like it may well one day be in every home". The same quiz book also offers intelligence tests—"use these tests to see how much quicker and brighter you are becoming, how you compare with your friends".

Home experiments seem to have become simpler and certainly safer with the years. No longer do you "hold the two handles of the induction coil and gradually insert the steel rod until you can no longer bear the tingling sensation. Compare your performance with that of your friends". But even a book of delightfully simple experiments has an oddity or two. Place a bottle between your mouth and a lighted candle. Why does the flame go out when you blow? "The air moving past the bottle creates a partial vacuum behind the bottle, and the air rushing in to fill this vacuum puts out the flame".

After junior science, the children can at least retreat into the unambiguous world of their first knitting kit—"Place a needle under your left arm. Hold the end of the yarn tightly in your left hand. Take the yarn coming from the ball into your right hand between your thumb and middle finger. Turn your index under the yarn between your hands towards you and point your index forward. There is a loop on your index". ☐

Articulating the aims of science

Nicholas Maxwell, of the Department of History and Philosophy of Science at University College, London, offers this comment on the current state of the art

MODERN science is, I suggest, seriously harmed by a widespread attempt to make science conform to a wholly inadequate philosophy of science—a philosophy of science which quite grotesquely misrepresents the basic intellectual aims of science.

This indequate philosophy of science may be called 'standard empiricism'. It is carelessly and unthinkingly taken for granted by the scientific establishment today. It is defended by almost all contemporary philosophers of science—from Popper to Kuhn, Lakatos, Hesse and Grünbaum—as constituting a rigorous, rational conception of science, even though elementary arguments show decisively that the view fails miserably to exhibit science as a rational enterprise.

The basic idea of this philosophy of standard empiricism is that the fundamental intellectual aim of science is simply to improve our knowledge of value-neutral factual truth, scientific progress being assessed in terms of how successfully this aim is being realised. *A priori* knowledge about matters of fact being denied, it is held that in science theories must be assessed, in the end, solely in terms of empirical success.

In fact, of course, science does not seek to improve our knowledge of factual truth as such; rather, quite properly, science seeks to improve our knowledge of *important* factual truth, truth that we deem to be in some way valuable, significant, interesting, beautiful, useful, from either a cultural or a practical, technological standpoint. Progress in science is assessed in terms of the amount of valuable factual truth that is being discovered: accumulation of trivia, however extensive, does not, and ought not, to be judged to amount to progress.

Once it is acknowledged that a basic intellectual aim of science is to discover valuable truth, it becomes clear that the aims of science must remain permanently and profoundly problematic. What is there to discover that is, in one way or another, valuable or useful? In what direction do really important, exciting future discoveries lie? What ought to be the general priorities of scientific research? What is at present of greatest importance or value, from a human, social standpoint? Important to whom? What kinds of things will we most need to know in fifty or one hundred years' time? These questions are all highly controversial and problematic just because they concern the domain of our ignorance, and difficult issues concerning human needs, aspirations and values.

It is of fundamental importance that we make the best possible choice of aims for science; and it is almost inevitable that we will fail. Here above all, then, we need to proceed intelligently, critically, imaginatively, wisely. If science is to proceed in a truly rigorous and rational fashion it is essential that we articulate, explore and criticise possible and actual aims for science as an integral part of scientific enquiry, providing ourselves with a wealth of critically examined alternatives, so that we may enhance our capacity to choose wisely and well. Quite generally one can say that rigour involves making explicit and so criticisable that which is implicit, influential and problematic. The best aims for science lie in the direction of the overlap between that which is scientifically realisable, and that which is humanly desirable. In order to give ourselves the maximum chances of discovering this problematic region of overlap we need to articulate both that which we conjecture to be scientifically realisable and that which we conjecture to be humanly desirable.

A truly rigorous, rational science would, then, include three kinds of contributions to science. In addition to contributions at the level of experiment and theory, we need to include contributions which articulate, explore and criticise possible aims for science. Scientific journals, textbooks, and educational courses need to include a discussion of all three kinds of contributions. It ought to be possible to win a Nobel prize by a sufficiently brilliant contribution at any of these three levels. We need to regard our scientific knowledge as consisting not only of what we know, but also of our best conjectures about what we do not know, but hope and desire to discover. We are never completely ignorant of that which we do not know; for if we were, we could have no basis for our assumption that the domain of our ignorance will not suddenly intrude upon the domain of our knowledge in some violent, unruly fashion, entirely disrupting the straightforward predictions of our present day scientific theories. Without some vague knowledge of that of which we are ignorant, all scientific knowledge would be impossible.

All this was well known to Einstein (*Ideas and Opinions*, Souvenir Press, 1973). He understood clearly that theoretical physics is only a worthwhile, rational enterprise when pursued as an attempt to discover a conjectured unified harmony in nature, the basic aims of physics thus being permanently and profoundly problematic. The success of Einstein's contribution to physics arose in large measure from his ceaseless concern to articulate and develop the best possible aims for physics, taking into account both existing knowledge and our desire to discover unity and harmony, appropriate heuristic rules then being formulated as guidelines to the development of new theories. I suggest that this kind of aim-oriented empiricist methodology, exploited with such success by Einstein within the restricted field of theoretical physics, is of universal relevance to science and technology.

At present aim articulation, as envisaged here, does not proceed as an integral part of scientific enquiry, just because most scientists take standard empiricism for granted. Once standard empiricism is accepted, scientific rigour and rationality actually seem to require that articulation and discussion of possible and actual aims for science be excluded from the intellectual domain of science. Thus, as a result of the attempt to make science conform to the intellectual ideals of standard empiricism, scientists have served to undermine the rigour and rationality of science, precisely because the vital task of articulating and exploring possible aims for science has been somewhat inhibited and repressed. As I have argued in greater detail elsewhere (*Phil. Sci.*, **41**, 123–53, 247–95 (1974); *What's Wrong with Science?*, Bran's Head Books, London, 1976), there is at present an urgent need for the scientific community to free science from the irrational ideals of standard empiricism, and to put into practice the kind of aim-oriented empiricist conception of science so successfully practised by Einstein. □

Gemona: the reckoning

An earthquake struck Gemona di Friuli, Italy, last May. **Nick Ambraseys** assesses its impact

THE Gemona earthquake of May 6, 1976, affected a large region in North-East Italy which, during the past nine centuries, has been devastated by a series of large shocks that have had recurrence intervals of almost total quiescence of 150 to 200 years. The largest of these events occurred on January 25, 1348, and on March 26, 1511. These earthquakes were associated with radii of perceptibility of 650 to 750 km and with aftershock sequences that lasted from three to six months. In addition, the shock of 1348 triggered a slide in the Gail valley, the volume of which exceeded 1,000 million m^3, a volume about four times larger than that triggered in 1963 at Vajont by the rapid draw-down of the reservoir level.

The Gemona earthquake occurred at 2100 hours local time near 46.24°N 13.28°E and had a focal depth of less than 10 kilometres; it had a surface-wave magnitude of 6.3 and was preceded by a single foreshock of magnitude 4.5. Another phenomenon which could have been premonitory was the appearance of a glow in the sky above the northern part of the epicentral region which persisted for some time after the earthquake.

There is no evidence that the earthquake was associated with surface faulting. Ground deformations originally suspected to be of tectonic origin in Montenars, Sedilis, Susans and Avasinis proved on examination to be of secondary nature. Persistent patterns of ground fracturing caused by slumping do not align with any minor or major fault.

The earthquake produced ground fracturing on flat and sloping ground, as well as a few cases of very local liquefaction. Landslides, rockfalls and debris-flows from steep slopes triggered by the shocks and heavy rains caused serious damage to property. Most of the rock faces and slopes that collapsed were already unstable.

More than three-quarters of the houses in the region are very old. Their walls consisted of coarse, short-bedded, badly-laid rubble masonry, sometimes mixed with bricks and with great thicknesses of lime or clay mortar joints, concealed by plaster or rough cast. Floors and roofs are heavy, resting on joists of timber. The use of tie-rods in masonry walls and laid at floor and roof level has saved many houses from collapse. New houses, mostly those built with some care after the local shocks of 1928 and 1939, have brick walls and reinforced concrete columns with cast-*in-situ* slabs. These houses, as well as structures built more recently with few openings and light roofs, survived the shock with comparatively little damage.

Both old and new houses which had recently been renovated suffered the most. A common cause of unintentional weakening was found to be due to the installation of electricity and water conduits in old houses, the re-location of bathrooms and sewer and drainage pipes in old walls, and the addition of extra rooms and floors in old dwellings. Damage to only less than a quarter of the houses in the region could have been prevented had modern houses been built to resist earthquakes; the rest were built long before the advent of modern technology. Lack of ductility and eccentric stiff structural elements in open ground floors were the main cause of damage to modern reinforced concrete houses and blocks of flats. Prefabricated constructions particularly in the industrial areas suffered more than other types of structures. The affected region was not considered to be highly seismic and very few buildings had been designed and built to resist lateral forces.

Within the region of maximum damage, intensities varied erratically from place to place and in only a few isolated points did they reach an intensity IX on the Mercalli scale. In this region the distribution of damage was influenced more by the type of structures shaken than by their foundation conditions. Intensities underground were much smaller than those experienced on the surface. In deep caves, delicately poised rocks, thin stalactites and stalagmites showed no signs of disturbance.

The maximum horizontal ground acceleration deduced from displaced objects in the meizoseismal region reached values of $75\%g$ associated with very high frequencies. The peak acceleration of the main shock recorded at a focal distance of about 30 kilometres (S—Start time=3.8 sec.) was $37\%g$ and the velocity 32 cm sec^{-1}. Peak accelerations of aftershocks of magnitude 5, recorded at smaller distances, reached values in excess of $50\%g$.

The main shock killed 965 and injured 2,286 people. The average rate of fatalities for the whole area is just under 1% of the affected population. All age groups except the infants and elderly suffered fatalities roughly proportional to their numbers. In densely populated villages and in historical town centres the more agile group of youths suffered more casualties in their attempts to seek refuge outdoors into narrow streets where they were crushed under falling walls.

The damage caused by the main shock was estimated late in May at $1,707 million. Damage to dwellings was $573 million, to agriculture $488 million, to industry $244 million, handicraft industry $146 million, public buildings $122 million, commerce $110 million, roads $16 million and aqueducts $8 million.

Preliminary estimates today for the total damage caused by the main shock and its aftershocks including loss of revenue from commerce amounts to $2,859 million. The total loss to buildings which includes 237,000 rooms of houses which were either totally destroyed or seriously damaged (excluding contents) has been estimated at $1,078 million. This figure is based partly on August–September 1976 market replacement values and partly on assessed

Destruction at Friuli

values. It includes replacement cost for 1,650 hospital beds and for seriously damaged or destroyed public buildings. Damage to industry, including handicraft and small industry is estimated at $599 million. Damage to agriculture amounted to $479 million which has now risen to $610 million.

Damage to public works and utilities amounted to $240 million including repair costs for the water supply, sewers, electrical facilities, aqueducts, road and railway communications. Loss of revenue from commerce is estimated at $240 million, and $176 million will be required for the repair and extension of the flood control of the Tagliamento valley. There is $49 million-worth of damage to school buildings and a sum of $96 million has been allocated for the repair of the few historical monuments that survived the shock. About 600 churches were destroyed or damaged beyond repair and damage to historical buildings, castles and works of art cannot be calculated.

One of the conclusions that can be drawn from the study of the Gemona earthquake is that this event could easily have been expected from what we know about the seismicity of the region This earthquake demonstrated once more the economic vulnerability of developed and densely populated regions of apparently low seismicity to strong earthquakes of long return period but short recurrence interval. It also demonstrated the need for the application in such regions of minimum earthquake resistant design requirements in the construction of new buildings and for the strengthening of old ones, particularly of historical monuments and art treasures. The amount and extent of damage caused by the earthquake and by its aftershocks have important implications for the insurance industry and for the social and economic planning in earthquake areas. □

The field work reported in this study constitutes part of the work carried out on a UNESCO Earthquake Reconnaissance Mission to Friuli (Ambraseys N., "The Gemona di Friuli (Italy) earthquake of May 1976", *UNESCO Rec Mission Report*, No 273 182 (1976), UNESCO, Paris). The writer would like to record his indebtedness to the President of the Regione, Dott A. Comelli, the Director of Forestry, Dott. R. Querini and to the Vigili del Fuoco for their generous cooperation.

Prediction: the negative aspects

The ability to predict earthquakes should reduce deaths, injuries, and property damage, but the prediction itself can have some negative effects. Henry Lansford sent this report from Boulder, Colorado, on a new study of the socioeconomic impacts of earthquake prediction:

WHEN social scientists go out into the real world to study a subject that has attracted a good deal of public attention, they usually come up with two classes of conclusions. The first class includes unsurprising findings that often provoke sceptics to snort that social science research is a waste of time—"common sense would have told you *that*!" But in addition to confirming some things that common sense might suggest, good social science research can turn up some surprises too—the unexpected insights into human behaviour that the sociologists like to call "counter-intuitive conclusions."

An 18-month study[*] of the potential impacts of earthquake prediction in the United States, conducted at the University of Colorado with support from the National Science Foundation, has produced both kinds of conclusions. The research team concluded, for example, that although the first credible predictions of damaging earthquakes months or years before they occur can save many lives and reduce property losses, such predictions can also result in severe economic depression and social disruption in the communities for which earthquakes are predicted. Although nobody had given much thought to this negative aspect of earthquake prediction, it should not come as a great surprise that property values and tax revenues will decline in an area for which an earthquake is predicted; that business activity, employment opportunities, and public services will be reduced; and that the sale of earthquake insurance policies will cease as soon as the prediction is issued.

But there are also some counter-intuitive conclusions. The researchers found few indications that homeowners who have small equities in heavily mortgaged homes would simply walk away and stop making their monthly payments, contrary to what common sense with a touch of cynicism might suggest They found that savings deposits in banks in the "target community" for which the earthquake is predicted would go up instead of down with the rest of the economy, as people save money instead of spending it on household appliances or other property that might be damaged or destroyed by the earthquake. And, perhaps most surprising of all, they found that 40% of the residents would remain, rather than evacuating either temporarily or permanently, in an area for which an earthquake five times as great as the one that devastated San Fernando, California, in 1971 has been predicted, even if they believe that the prediction is accurate.

The research team, which included both physical and social scientists, did most of their field work in California, which has had more recent experience with earthquakes, as well as more trepidation about future ones, than any other part of the United States The team also looked closely at earthquake-prediction research that has been going on in China, Japan, and the Soviet Union. They began by getting estimates from seismologists of the probable characteristics of the first official, scientifically-based earthquake prediction in the United States and of the way in which it would probably be released to the public. Then they formulated two "prototype" predictions: one assumed a five-month lead time, a 50% probability of occurrence, and an earthquake with a magnitude of 6.3 on the Richter scale; the second assumed a three-year lead time and a 7.3 magnitude. Then they conducted more than 1,000 interviews throughout California, with federal, state, and local government officials, executives of large and small businesses, managers of large and small news media and individual families.

Three of the study's findings are regarded as particularly significant:

- Under existing laws, it is not clear whether federal disaster-relief funds can be committed on the basis of an official earthquake prediction or whether it will be necessary to wait until earthquake damage has occurred before committing funds that might have helped prevent the damage
- No workable alternative to earthquake insurance exists for indemnifying those whose property is damaged or destroyed by an earthquake, although the California state insurance commissioner has stated that he will stop the sale of new earthquake insurance policies in any area for which a credible official earthquake prediction has been issued.
- Damage estimate maps, based on solid scientific data and published by news media in the threatened area, should reduce property damage considerably if the earthquake prediction provides enough lead time to reinforce structures and undertake other damage-prevention measures. □

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[*] J Eugene Haas and Dennis S Mileti, *Socioeconomic Impact of Earthquake Prediction* (Institute of Behavioural Science, University of Colorado, Boulder, Colorado; 1976)

USSR

What sort of turning point?

Vera Rich reports on the fate of dissident Soviet scientists in light of the recent release of Vladimir Bukovskii.

THE release of Vladimir Bukovskii, in "exchange" for the Chilean Communist leader Luis Corvalan, brings to the West one of the most significant figures in the human rights movement in the Soviet Union. It does not, however, appear to indicate any general amelioration of the plight of dissidents; indeed, following Bukovskii's release, a number of other dissidents and refusniks, including several prominent scientists, were arrested.

Bukovskii, who was first arrested in 1963 while still a student of biology, has spent ten of the last thirteen years in penal psychiatric hospitals, prisons, and strict regime labour camps. During one of his brief periods of freedom he managed to inform the outside world of the growing Soviet practice of employing psychiatric methods to repress political dissidents, thereby posing an ethical problem to Western psychiatrists as to what should be their professional relationship (if any) with those of their Soviet colleagues known to be involved in this abuse. Imprisoned once again, in 1974 Bukovskii was sent to the strict regime labour camp at Perm', where with his fellow prisoner, the psychiatrist Semeon Gluzman, he wrote the now-famous *Manual of Psychiatry for Dissidents,* which sets out self-defence strategies for those whose political or ethical convictions are judged by the authorities to be evidence of insanity.

Immediately on his arrival in Zurich, Bukovskii thanked those who had campaigned for his release and announced his intention of devoting the rest of his life, if necessary, to campaigning for his fellow prisoners-of-conscience. In the euphoria of release, he said that he felt his liberation marked an important new trend in Soviet policy and was "a great victory for everyone". This hope, however, does not appear to have been justified. The "exchange" procedure which brought Bukovskii freedom has been tacitly denied by the Soviet authorities; officially, Corvalan was released due to the efforts of "progressive forces" throughout the world, while Bukovskii was expelled from the Soviet Union as a terrorist. And, a few days after Bukovskii's release, another former long-term psychiatric detainee, the electrical engineer Vladimir Borisov, was rearrested.

Borisov, who was released in 1974, had spent the major part of the previous eleven years in penal psychiatric hospitals for having organised an unauthorised youth group. During his captivity, he took part with Viktor Fainberg in a hunger-strike to protest against the conditions under which the prisoners are kept and the denial of their basic rights. Borisov's rearrest, on December 25, was allegedly in connection with the anti-Soviet slogans which appeared on walls in Leningrad earlier this year; dissident sources in Leningrad, however, interpret it as a warning from the authorities not to assume that Bukovskii's release marks a new 'thaw'. Borisov is at present, confined in a Leningrad psychiatric hospital in a block for 'acute' and violent cases, and has not yet been seen by the 'commission' of three psychiatrists which, legally, must decide on his case. His wife Irina is demanding that the commission should consist of psychiatrists whom she can trust, and has asked for Semeon Gluzman, Marina Voikhanskaya, and Gary Low-Beer.

This request is unlikely to meet with official approval. Dr Gluzman is still in the Perm' labour camp, where he was sent for exposing the 'official' diagnosis of insanity in the case of General Grigorenko. Dr Voikhanskaya, who formerly worked in the hospital where Borisov is now confined, was obliged to emigrate (leaving her young son behind) after her repeated refusals to administer psychotropic drugs to dissidents. Dr Low-Beer, of Horton Hospital, Epsom, a prominent campaigner against the political misuse of psychiatry, told *Nature* that he is quite willing to comply with Mrs Borisova's wish that he should examine her husband, and that although it is virtually impossible that the Soviet authorities would allow such a commission to decide Borisov's fate, he himself would press for and demand the right of access to the 'patient'.

Unlike Borisov's arrest, the arrest and harassment of some 45 Jewish refusniks in Moscow (including the physicists Mark Azbel and Aleksandr Lerner, the mathematician Viktor Brailovskii, and Academician Veniamin Levich, an electrochemist) is not directly associated with the release of Bukovskii. The group, members of the now-famous Sunday seminars for dismissed scientists, had been under pressure for some weeks following the announcement of their intention to hold a symposium on Jewish life and culture in the Soviet Union. (A growth of Jewish consciousness is, not unsurprisingly, a common phenomenon among the refusniks).

Vladimir Bukovskii

The group had been warned that if they persisted in going ahead with their plans, the authorities would take action. The threat was reinforced by a vicious attack in *Izvestiya* on Aleksandr Voronel, the founder of the group, who emigrated to Israel in December 1974; this called him a "former scientist without a future, indeed without a present", a "trader in human souls" and an "unmasked provocateur". The article recalled how, in summer 1974, Voronel's efforts to hold an international session of the seminar "failed completely"—as indeed it did, since would-be participants from abroad were refused visas and the "hosts" were arrested or subjected to severe harassment. A repetition of these tactics in the case of the Jewish symposium was therefore expected; it was hoped, however, that the authorities would intervene simply to break up the symposium, and that there would be no long-term arrests.

This, unfortunately, has not proved to be the case. Criminal proceedings have commenced against one of the would-be participants, the physicist Naum Salanskii from Vilnyus, on the grounds that the paper he had prepared for the symposium slandered the Soviet Union by giving incorrect information about the treatment of the Jewish minority. In Leningrad, a Jewish mechanical engineer, Vladimir Shverdlin, was arrested on a charge of unlawful possession of weapons (apparently a few bullets left over from his membership, some years back, of a shooting club); although he has since been released, his family have been informed that the results of the investigation have been "passed on to the Procurator".

In spite of such warnings, the human rights campaign still continues. Following the arrest of Borisov, a group of eighteen Moscow dissidents has called for a renewed campaign against the political abuse of psychiatry. In Kiev, a 'Helsinki monitoring group' has been set up, similar to that set up earlier this year in Moscow; its members include Nina Strokata-Karavans'ka, a microbiologist, and the science-fiction writer Mykola Rudenko. □

USA

● President Ford's final budget, which is expected to be delivered to Congress on January 17 (just three days before his successor is inaugurated), will promise large increases in funds for research and development and propose that the green light be given to several important and eagerly-awaited scientific projects. Though it's possible that the whole budget document could be turfed out by the incoming Carter Administration, Mr Carter will have more pressing matters on his mind than tinkering with the nuts and bolts of the federal government's science programmes; it is therefore likely that some of Mr Ford's lame-duck proposals will survive more or less intact.

The rough outline of the proposed budget for research and development was sketched out last month by Guy Stever, the President's science adviser, who is also about to leave the government. Stever called a press conference after meeting to discuss the budget with Mr Ford, a number of prominent people from the scientific community and government officials from science agencies. He announced that Ford will propose that basic research be given a real increase, amounting to about 3% above the rate of inflation, in the fiscal year which begins on October 1, 1977, and that some of the increase would be spent on new projects in agricultural research, space science and earthquake prediction.

Following the pattern established in his two previous budgets, President Ford will propose particularly large increases in military science and technology and energy research and development. The proposed increase in military science alone will amount to about 15%, Stever said. Carter will take a close look at both those two areas, however, and Ford's proposals should therefore be taken with more than a pinch of salt.

As for space science, the proposed budget will contain some good news for astronomers and planetary scientists. Last year, in an effort to reduce public expenditure, the Ford Administration stripped about $200 million from the budget of the National Aeronautics and Space Administration shortly before it was sent to Congress, and NASA was consequently forced to defer plans to build the Large Space Telescope (LST) and to start work on a spacecraft to orbit Jupiter. Those funds will be reinstated in this year's budget, however, and if approved by the Congress and by the Carter Administration, the LST should be ready for launch in the early 1980s and the Jupiter mission will be launched in 1981. Both missions rank high on the list of priorities for space research drawn up by the National Academy of Sciences.

Another space project which Ford will approve is construction of a fourth satellite in the Earth Resources Technology Satellite series, called Landsat-D. The Landsat programme has consistently received strong support from Congress, and Ford's proposals will probably be accepted.

The lame-duck budget will also include a large helping of money for earthquake research, another area in which Congress has taken an interest. President Ford will propose that funds for earthquake monitoring and prediction be doubled in the next fiscal year, to reach about $50 million. The money would be divided between the US Geological Survey, which operates a number of seismic stations and research establishments in California, and the National Science Foundation. No details are available of the projects which will benefit from this largesse, however.

Finally, in agricultural research, President Ford has decided to implement a proposal which has been advanced by three committees of the National Academy of Sciences and by two committees of the House of Representatives. He will propose the initiation of a new programme of competitive grants, funded through the Department of Agriculture, for basic research related to agriculture. The new programme, which would receive about $35 million next year under Ford's proposal, will complement rather than replace the Agriculture Department's traditional block grant system for agricultural research.

Usually, budget details are not released until the proposals are sent to Congress, but in this case, the tradition was broken presumably because the figures for research and development look good. Since Guy Stever and the Office of Science and Technology Policy can claim a good deal of the credit, they were understandably keen to get the word out before Stever departs.

● The National Academy of Sciences has finally added its voice to the chorus of criticism directed at the so-called 'hot particle' theory. Put forward in 1974 by Drs Thomas Cochran and Arthur Tamplin, staff scientists at the Natural Resources Defense Council, the theory is that tiny particles of inhaled plutonium may lodge in the lung and deliver a prolonged, intense dose of radioactivity to a small area of surrounding tissue, thereby posing a severe cancer hazard. NRDC argued that since present plutonium exposure standards are based on average doses of radioactivity to the entire lung, they underestimate the health hazards and should be tightened by a factor of about 115,000. Such strict standards would present a problem for reprocessing and plutonium fabrication plants.

In a report published last month, however, an Academy committee concludes that the evidence doesn't support the hot particle theory and that there is no need to lower plutonium exposure standards. The committee, which examined the theory under contract to the Environmental Protection Agency, looked at the results of tests on beagle dogs and concluded that the observed incidence of cancer doesn't fit in with the hot particle theory, and suggested that it "can adequately be accounted for by averaging the absorbed alpha radiation dose over the whole lung." The committee also argues that epidemiological evidence from experience with inhaled alpha-emitting particles suggests that the usual method of applying an average radiation dose to the whole lung to predict carcinogenic effects.

Colin Norman

FRANCE

Deceptively generous budget

The staff of La Recherche *report from Paris on the funding for research in France in 1977*

THE National Assembly and Senate of the French parliament have just approved the budget for scientific research for 1977—that is, they have approved the provisional calculations of expenditure for the 'enveloppe recherche' presented by the Minister for Industry and Research. But this budget package, since it does not take into account military research or telecommunications, is only a fabrication.

As early as the end of May last year, the first draft of the 'enveloppe' had been strongly criticised by the consultative Committee on Scientific and Technical Research. M d'Ornano had then managed to get some adjustments to it through the Ministry of Finance. He finally obtained 10,935 million francs, an increase of 18% on last year's budget of 9,262 million francs. The major portion of this sum goes as usual to the Atomic Energy Commission (FF2,783 million), the National Centre for Space Research (FF1,014 million) and to information processing (FF725.6 million). The National Centre for Scientific Research receives FF2,555 million.

The budget is larger, however, only because more jobs have been created. And it must be remembered that the package is already cut into by international commitments and by other expenditure unconnected with research. For the National Centre for Space Research, for example, FF586 million is reserved for multilateral programming, such as the programmes operated within the European Space Agency like Ariane, Marots and Spacelab. For information processing, on the other hand, FF450 million is allocated to CII–Honeywell-Bull.

The government has decided to create 950 new posts, of which 437 would be for research, and the rest technical or administrative. Furthermore, 900 people, 95 of them research workers, will benefit by the integration of 'hors statut' personnel. (Staff who

ISRAEL

• Israel's universities have spawned a new political party which threatens the local establishment more seriously than it has been threatened in many years. If present trends continue, the recently formed Democratic Movement of the Hebrew University archaeologist Yigael Yadin, and other reformist groups close to it, may well emerge as the strongest bloc in Israel's Knesset after the 1977 elections, perhaps even making it possible for Yadin to become the next Prime Minister. The elections are being held sooner than expected with the early end of Mr Rabin's government.

Yadin offers very little that is new in the policy sphere. Like large sections of the hitherto dominant Labour Party, he favours a compromise settlement with the Arabs, electoral reform and stringent measures to stabilise the economy. But he has the advantage of being a political outsider, untainted by the corruption and dissension that has beset Israeli public life in recent years.

This is not to say that Yadin has been an academic recluse in the quarter century since his retirement as Israel's second chief of Staff. On the contrary, his highly successful digs at Hazor in the Galilee and at Massada on the Dead Sea were well publicised, as was his service on such non-partisan bodies as the Agranat Commission (which investigated aspects of the Yom Kippur War). This makes him particularly attractive to the many Israelis who ardently desire "to throw the rascals out", but want to be sure that "the rascals" are replaced by men of experience and competence.

Yadin is by no means the only Israeli don to have played a part in public life: Israeli's first President, Dr Chaim Weizmann, was a distinguished chemist, and her current President, Professor Ephraim Katzir, headed the Weizmann Institute's Biophysics Department for many years. But the Presidency is largely a ceremonial post, and this is the only time that a savant has tried to exchange his academic robes for the powerful mantle of the Prime Minister.

• A central plank in Yadin's platform is his insistence that Israel remain a predominantly Jewish state, meaning that many of the Arab-populated areas now under Israeli control would, if he had his way, be relinquished within the framework of a peace settlement. This being the case, Yadin favours concentrating long-range development efforts on those parts of the country where the Arab population is sparse, with particular emphasis on the virtually empty Negev desert between Beersheba and Eilat.

Thanks to the discovery of a vast underground lake running from the Dead Sea to the middle of the Sinai Peninsula, it now appears that the Negev can be developed more rapidly and extensively than hitherto appeared possible. The Mekorot Water Company has carried out drillings in the wake of preliminary studies by scientists from the Ben-Gurion University of the Negev and the Weizmann Institute. Company officials speak of at least 200,000 million cubic metres of water at depths of between 800 and 1,000 metres. Initial tests indicate that the water has from 500 to 2,000 p.p.m. of minerals, which means that some of it can be utilised immediately, while other water will have to be processed through reverse osmosis or electrodialysis installations.

Water from the Negev 'lake' must be exploited carefully, as it is not likely to be replaced for quite some time. Most of it, according to carbon-14 dating tests, stems from the precipitation that occurred 20,000 years ago during the last Ice Age, when rainfall in the Negev averaged 1,000–3,000 mm a year, as compared to some 100 mm today.

• Rainfall now, it turns out, is heaviest where it is least needed, in Israel's urban areas. A recently completed study of climatic conditions in the Tel Aviv area over the last 30 years carried out by Dr Yair Goldreich of Bar-Ilan University and Alexander Mannes of Israel's Meteorological Service showed that rainfall had increased by 5–17% as compared to the rest of the countryside.

This study indicated that heat generated by greater radiation from concrete, asphalt and other built-up surfaces weakened the frequent temperature inversions of the air above the city, creating unstable conditions conducive to rain. At the same time, the greater currents of air caused by this heat and also by tall buildings contributed further to increased precipitation.

Another conclusion of the study was that growing air pollution in the Tel Aviv area, where population has tripled in the past 30 years, had caused a drop of 7% per decade in the amount of solar radiation reaching the ground.

Nechemia Meyers

are 'hors statut' are employed on contract for a limited period, whereas usually, in 'la recherche publique', researchers have the status of a civil servant, and are therefore guaranteed tenure).

There is also to be a large increase in the funds allocated to university research, due to go up from FF76 million in 1976 to FF120 million in 1977. This increase will rekindle the enthusiasm of the professors, who in return are urgently asked to put the affairs of the State Secretariat for Universities in order.

The research priorities of the Seventh Plan are, of course, the best safeguarded. Among these is research to reduce France's dependence on other countries for energy and basic materials. So FF195 million will be spent on providing new energy sources, and FF45 million on uranium prospecting. In addition, special efforts are to be made to improve food production, scientific and medical instrument manufacture, prevention of pollution, and cooperation with underdeveloped countries.

It is indeed a budget which is misleading in its generosity, but which follows well-marked political intentions. □

SOUTH AFRICA

●South Africa is to embark on the construction of a 200 MeV open sector cyclotron early in 1977, thanks to the award of a government grant of R570,000 (£1=R1.45) for the next stage of the project. This is in addition to the more than R200,000 already spent on feasibility studies.

The facility, which will be built near Cape Town, has been 4 years in the planning and is expected to be operational in 1983. In due course the cyclotron and its staff will absorb the functions of the present Southern Universities Nuclear Institute at Faure, and progressively replace the CSIR's cyclotron, which is expected to have a lifetime of only a further 6 years. The new cyclotron will be used primarily for the production of radioisotopes for medical research, for radiotherapy, and for nuclear physics research.

Responsibility for the facility will be vested in an independent company, still to be set up, the funding for which is likely to come directly through the office of the Prime Minister's Scientific Adviser.

●The Prime Minister's Scientific Adviser, Dr S. Meiring Naudé, will retire at the end of the year. His successor is to be Dr A. P. Burger, a former director of the National Research Institute for Mathematical Sciences and currently a vice-president of the CSIR. Dr Naudé, a former president of the CSIR and one of South Africa's most respected scientific administrators, oversaw the introduction in 1973 of the procedure which is now followed for funding government-supported scientific research in South Africa. All requests for research grants are now channelled through the office of the Scientific Adviser, who alone pleads the case for state support for science with Treasury. Major research organisations in the public sector submit estimates for the coming year for both ongoing research and new projects.

Recent practice has been to award an across-the-board increase of at least 6% in grant aid to all applicants mostly for ongoing research. As a means of exercising control on the direction of the national research effort, however, approximately 15% of the funds available have been assigned at the discretion of the Scientific Adviser to support projects recognised as being in the national interest. By this means, agricultural research, which a few years ago was automatically given more research money than it had the resources to use effectively, now receives about 32% of the total compared with nearly half four years ago. In contrast, the budget of the National Institute for Metallurgy has been growing at about 25% annually, and that of the Human Sciences Research Council by at least 15%. Overall the national R&D budget has been growing by about 11% annually.

To help him in this sensitive task, Dr Naudé made use of an independent committee to set research priorities, consisting principally of eight senior scientists. Some were retired and others were attached to industry; none had a direct interest in the research organisations seeking support.

●Until it was closed down earlier this year, the deep space tracking station operated by the CSIR at Hartebeesthoek, 40 miles from Pretoria, had earned a reputation for being one of the most reliable of NASA's worldwide network of satellite tracking facilities. Far from going out of business, the team of skilled engineers and excellent technical facilties built up during that period will now provide the basis for converting Hartebeesthoek into a monitoring station for remote sensing satellites.

The deputy president of the CSIR, Dr F. J. Hewitt, recently announced that plans are in hand to establish a line scan image processing facility service in conjunction with, initially, the Meteosat and Landsat satellites. When it becomes operational, Meteosat will play a crucial role in monitoring weather conditions over Southern Africa and the adjacent oceans. As for Landsat imagery, the new facility with its sophisticated electronic processing equipment will make it possible to collect remotely sensed data of much higher quality than is presently available from NASA in the form of transparencies of images recorded in different spectral bands.

The CSIR has become concerned that, in Southern Africa, too little advantage has so far been taken of what remote sensing satellites have to offer and, recognising that they are here to stay and bound to develop, is now to make a major effort to provide whatever facilities of hardware and software are needed to keep abreast of satellite technology. The CSIR will accept responsibility for the development of the more fundamental image processing methods, as well as for the provision of the usual infrastructural services at the tracking station. It is expected, however, that users will in time develop procedures for their own particular problems. Encouragement will also be given to sharing the facility with neighbouring territories, four of whom are already actively involved or interested in remote sensing.

Hewitt hopes that the station would be operational by August of next year, and that the sophisticated data processing and information extraction service will be available early in 1978.

Graham Baker

IN BRIEF

JET in difficulty

The hoped-for meeting of the EEC's Council of Research Ministers, provisionally scheduled for the week before Christmas, did not take place once it became clear that the Nine would again fail to agree on a site for the Joint European Torus (JET) project. The main obstacle seemed to come from France, which apparently intended to veto any proposed site other than Cadarache and suggested only CERN as an alternative. No date has been set for a future meeting. The view of the outgoing Research Commissioner Guido Brunner, that JET is "on its deathbed", is not accepted in Britain, where it is seen as an attempt to encourage an early decision.

Reprocessing developments

West Germany's recent indication that she will follow France and stop exports of nuclear reprocessing technology will not apparently cover her controversial deal with Brazil. France's own policy leaves unaffected her proposed export of a reprocessing plant to Pakistan, which is resisting pressure to cancel the deal.

Canada has meanwhile ended nuclear cooperation with Pakistan, as she did with India earlier last year, as part of a new exports policy just announced. In Britain the UK government has decided to subject plans for an oxide fuel reprocessing plant separately to a full public inquiry, but plans to expand facilities for Magnox fuel should receive an early approval. Elsewhere, Argentina has claimed she now has the wherewithal to produce nuclear weapons.

Research priorities decided

Specific areas of research in the field of gastroenterology are to be given priority by the UK Medical Research Council and the Department of Health and Social Security. Problems earmarked for the next 5 years include peptic ulcers, inflamatory bowel disease, infective hepatitis, cancer of the intestinal tract and gallstones; priority is also to be given to the study of gastrointestinal hormones, nervous control of the gut and the fundamental mechanisms of both intestinal absorption and pancreatic secretion.

Shetland's oil

KENNETH MELLANBY

THE discovery and development of North Sea oil is already having a profound economic and social effect in those parts of Scotland where it is beginning to be brought ashore. Money is pouring in unprecedented amounts into areas which have always been poor. The extraction of oil is expected to reach its peak in the 1980s, and the reserves may be exhausted before the end of the century. While I tend to share the pessimism of the recent Nobel Laureate Milton Friedman regarding the probable effects of the oil on Britain's flagging economy, I am most concerned about the ecological effects of the industrial developments, and the possibility of pollution by oil spills, breaks in the pipelines and even by continuous low level seepages on the wildlife of many parts of the Scottish coast.

One area most likely to be affected is the Shetland Islands, the most northerly group of the British Isles, where gales and high seas make the work particularly hazardous. This hitherto undeveloped region, with a population of only 19,000, will have a terminal which will deal with as much as 150 million tonnes of crude oil in a year, more than the present total imports of mainland Britain. The likely results are summarised in a small book, *The Shetland Way of Oil* (Thuleprint, Sandwick, Shetland; 1976), produced by a group of mainly local writers. They describe without undue sentiment the way in which a life-style still cherished by at least the older Shetlanders is doomed, and suggest how permanent damage to the islands could be minimised. They are particularly aware of the limited volume of the oil reserves, and the need to exploit them rapidly to repay the enormous capital invested. They wish to prevent a temporary boom declining rapidly and leaving ghost towns and derelict installations in a devastated environment.

The oil companies appear to be acting responsibly, and to be cooperating in trying to alleviate any unavoidable damage. The cynical will no doubt suggest that this is in order to placate local interests which could delay development, but nevertheless the results are promising. The UK parliament has passed the Zetland County Council Act (1974) which gives this small local government authority financial powers rather greater than those proposed for the Scottish Assembly, and they should eventually have a very large sum of money, perhaps more than a hundred million pounds, to restore damage and provide industries and amenities when the oil is exhausted. It is thus likely that only the minimum damage will be done to the landscape, to buildings and to antiquities, and that means will be found to encourage other industries to absorb those unemployed when the oil boom ends.

It is difficult to be so optimistic about wildlife, for in an operation of this size some serious pollution is almost inevitable. The good thing about the oil rush is that it has made conservationists aware of the richness of the flora and fauna of Shetland and of Scotland generally, and of their ignorance of what is actually there. It has stimulated ecological surveys by and on behalf of the Nature Conservancy Council, which show us what might be lost. Plans are being made to monitor any changes which occur during the exploitation of the oil, so that damage may be quickly assessed, even if it cannot always be prevented. Attempts are being made to find ways of minimising any damage if leaks or spills occur. Some surveys were not completed soon enough to prevent shore works being carried out in valuable wildlife areas, but some attempt to avoid these has been made. The problem of the ultimate rehabilitation of damaged habitats will also be studied.

Fishing, farming and knitting are traditional industries of Shetland. Already oil has contributed to the reduction in fish caught, though overfishing and international politics have also had their effect. High wages at the oil terminal take labour from the farms and the knitting factory. It is hoped that, when the oil is exhausted, these and other industries will be restored and developed. If there is a world energy crisis there should be an assured market for Shetland knitwear, including the Fair Isle pullovers so popular in the 1920s with the then Prince of Wales, to keep us warm when we can no longer afford to overheat our buildings.

correspondence

The scientific civil service

SIR,—Your leading article of November 18, "Don't Move", is itself guilty of throwing "the same old accusations" at the scientific civil service without any supporting evidence. The IPCS would certainly agree that the scientific civil service still lacks the right openings for mature scientists whether from within or without. It cannot, however, accept that the scientific civil servant is considerably better paid than his counterpart elsewhere; nor do scientists in the civil service feel particularly secure in their jobs at a time when cuts in public expenditure are raising real threats of redundancy.

It is of course true that industrialists frequently complain that they find it difficult to recruit in competition with the service; they are less willing to make public the details of the terms they themselves offer to scientists so that comparisons can be fairly made. Meanwhile the trend away from science continues in both the schools and the universities. The recent report from the Select Committee on Science and Technology, "University–Industry Relations", underlines the importance of reversing that trend. It will not be done by continued sniping at particular employment sectors but by improving the status of engineers and scientists in the community at large and, as the Select Committee recognises, by giving them "the opportunity of moving into the top echelons of management with as much ease as their counterparts in Germany, France or the USA and as their counterparts in Great Britain who have chosen to study law or accountancy".

Greater movement of scientists between different sectors of employment, though desirable, will not of itself solve the underlying problem of the unattractiveness of science as a career—whether in industry or the civil service.

MARGARET PLATT

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International contacts

SIR,—I would like to complete Professor Burhop's article ("A case of suitable treatment?", November 11) by one relevant remark. At the same time as London created obstacles in issuing British entry visas for some delegates of the World Federation of Scientific Workers' General Assembly, the former vice-president of the Federation and a long term head of its Prague Regional Secretariat, Academican Ivan Málek, was visited by two gentlemen from the Prague police, who confiscated his foreign passport, valid only for East European countries—perhaps from fears that he might try to participate in the Pugwash meeting in August in East Germany, or the London Assembly.

I fully understand Professor Burhop's indignation about the bureaucratic obstacles to realising international scientific contacts. What I don't understand is why he does not publicly condemn equally strongly the incomparably heavier fact that thousands and thousands of scientists from the USSR and Eastern European countries are extremely restricted in travelling abroad, that every single journey must be approved and depends fully on the goodwill of the authorities and political views of each applicant?

Professor Málek, a scientist with an international reputation and a member of many international organisations, committees and boards, has not been allowed to leave Czechoslovakia for over 6 years (although he is constantly being invited abroad), and only because of his disapproval of occupation of Czechoslovakia in 1968 by Soviet and "allied" armies.

FRANTIŠEK JANOUCH

Stockholm,
Sweden

USSR river diversions

SIR,—There is a disturbing aspect of the Soviet plan to divert the rivers Irtysh and Ob southward (December 2, page 390) which your correspondent does not mention. Calculations reported by Aagaard and Coachman in *Eos* (**56,** 485) suggest that by removing or reducing the low salinity layer at the surface of part of the Arctic Ocean such a scheme could allow deeper convection of the surface waters, encouraging ice to melt with perhaps a positive feedback as the albedo of the region is thereby reduced. Likely results, they suggest, "would be (1) prolonged ice-free conditions because of deep-reaching free convection . . . (2) the release of large amounts of heat from warm Atlantic water during the cold months, and (3) the elevation of quite saline water into close proximity with the sea surface".

It is not clear, of course, whether such effects should be regarded as desirable or undesirable; in the present state of the art regarding our understanding of climatic changes we can only say that repercussions of one kind or another on the general atmospheric circulation would be inevitable. Given the uncertainties involved, however, it seems most unwise to carry out the experiment.

JOHN GRIBBIN

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Competition 11

Readers are invited to submit descriptions, in epigrammatic reportorial form, of the demeanour, condition or action of any of the famous scientists (past or present) participating in an imaginary general symposium. Submissions should offer some humorous association with the scientists' work, for example, "Faraday was incapacitated", "Boyle and Charles felt the meeting was 'a perfect gas' ". Closing date for entries: February 16. (Competition inspired by Charles S. Cope of Parkersburg, West Virginia.)

Competition 10. "Prehistoric camels often sit down carefully. Perhaps their joints creak. Early oiling might provide permanent relief". These memorable phrases earn Dr P. Little, of Wantage, UK, £10 as the best mnemonic of a rather disappointing bunch. They remind us that the geological epochs are Precambrian, Cambrian, Ordovician, Silurian, Devonian, Carboniferous, Permian, Triassic, Jurassic, Cretaceous, Eocene, Oligocene, Miocene, Pliocene, Pleistocene and Recent (with apologies to the Paleocene).

As we said to our accounts department, "Prepare cheque of suitable denomination. Competitor pleases the judges, conquering easily over many people's poorer reminders".

news and views

Interpreting Stonehenge

from R. J. C. Atkinson

THE formulation of astronomical interpretations of Stonehenge is becoming a minor industry with its own characteristics. These are well illustrated by a recent and a current contribution to *Nature*.

R. F. Brinckerhoff has suggested (*Nature*, **263**, 465; 1976) that selected depressions on the tops of some of the lintels were made to hold markers used by an observer, himself standing on the lintels on the opposite side of the circle, to identify significant risings of the moon. This is clearly possible under certain circumstances; but is it likely?

I think not, if only because the tops of the lintels are now so weathered as to make it impossible to distinguish artificial holes from those of natural origin. On other lintels, notably stone 152, there are many weathered holes far deeper, and no less regular in outline, than Brinckerhoff's examples, for which no astronomical explanation is possible. Furthermore, the author admits that the relevant observations could have been made only after the fall of stone 55. The date of this event is unknown; but we do know from Gowland's excavations of 1901 (*Archaeologia*, **LVIII**, 37–118; 1902) that the base of this stone was not deliberately undermined. The differential weathering of the upper and lower surfaces of the lintel which it formerly supported (stone 156) suggests that stone 55 may not have fallen until well after the end of the Early Bronze Age. There is no archaeological evidence for the continuing use of Stonehenge thereafter.

A. D. Beach (this issue of *Nature*, page 17) makes the new, ingenious, but to me equally improbable suggestion that the Aubrey Holes were used as counters for predicting tidal amplitudes by way of previous knowledge of the period of rotation of the lunar apsides. This is a more 'difficult' explanation than Hoyle's (which Beach apparently rejects) because it involves an additional generalisation from natural phenomena unobservable at Stonehenge itself.

From our present knowledge of celestial mechanics it is all too easy to impute to our prehistoric ancestors an understanding of nature which may well be ours alone. Both these papers, by concentrating exclusively on the astronomical interpretation of aspects of a monument which was surely more than an observatory (if it was ever that), lead to invalid inferences and to the posing of non-problems.

Brinckerhoff says, for instance, "No one has ever explained convincingly why it [the linteltop walk-way, so-called] had to be 16 feet high." Why indeed, except that the builders so decided within the limits of the building material available? Has anyone, for that matter, explained convincingly the height of the spire of Salisbury Cathedral? That the lintel-tops could have been used as an elevated walkway does not necessarily imply that they were.

Beach, in advancing a purely 'functional' purpose for the Aubrey Holes, dismisses the 'religious' explanation of Stonehenge I as "surely the lowest common denominator of imprecision and evasion". One wonders how he accounts for the use of Stonehenge I as a cemetery, and for the occurrence of similar cremated burials in similar rings of pits elsewhere in Neolithic Britain, for which no 'functional' purpose has ever been suggested. In fact, of course, one need look no further than many Christian churches, orientated on the sunrise either at the equinoxes or the patronal saint's day, to see that a religious purpose need not be incompatible with some elements of astronomy; nor does the incorporation of sun-dials and clocks in churches imply that they were built primarily as timepieces.

Both these papers seem to me to show an almost obsessive desire to interpret Stonehenge exclusively in 'practical' or 'scientific' terms, and to ignore the probability that it served simultaneously a number of purposes which only we would regard as separable or conflicting, and many of which, could we know them, we should find it virtually impossible to understand. The distinctions which we make between the rational and the irrational, the practical and the useless, or between religion and science, are all part of a universe of discourse which is quite inapplicable in a prehistoric context. To impose that universe of discourse on our remote ancestors is to abolish history, and to people the prehistoric past with ourselves in fancy dress. □

Metal bites metal

from Robert W. Cahn

THE recent Report of the Court of Inquiry (HMSO, London, 1975) on the disaster at Flixborough, where a caprolactam manufacturing plant exploded with grave loss of life, cited surprising evidence concerning weakening of hot stainless steel by traces of metallic zinc on the steel surface. This had not been recognised before the disaster inquiry and the special research commissioned to help in that inquiry. It appears that minute quantities of zinc, which can reach the steel either as liquid or as vapour, lead to fracture within seconds at modest stresses (5–6 kg mm^{-2}) in the temperature range 800–900 °C. Without the zinc, a stress well over 10 kg mm^{-2} would be needed in this temperature range to achieve similarly rapid fracture. This is an instance of stress-corrosion, a phenomenon which involves the mutual abetment of stress and corrosion: the corrosive agent accelerates the action of the stress and the stress enhances the corrosive attack. The importance of zinc-embrittlement in the sequence of events that led to the explosion was the central and highly disputatious issue at the Court of Inquiry; mutterings of disagreement as to the official findings may still be heard today.

Stress-corrosion by liquid metals takes place far more commonly than was recognised two decades ago: the subject has been comprehensively reviewed by M. H. Kamdar (*Prog. Mater. Sci.*, **15**, 289–374; 1973). Generally, such attack is not pronounced at or near ambient temperature, and it is thus a form of brittle fracture, enhanced by adsorption of the contaminant at the root of surface cracks. Some trace metals embrittle a variety of solid metals: thus mercury embrittles aluminium, zinc, cadmium, iron, silver, titanium and others, gallium attacks most of these metals (most spectacularly, aluminium), whereas bismuth only embrittles copper, and cadmium only iron and titanium. Mercury can claim championship status as a general trace nuisance in biology and engineering alike!

A paper by W. T. Grubb in this issue of *Nature* (Grubb, page 36) adds a further example to the growing number of instances of liquid metal embrittlement. It resembles the stainless steel/zinc syndrome in that the embrittlement is specific to high-temperature stressing and does not arise at room temperature, unlike the general run of such cases. Grubb has discovered that zircaloy-2 (a zirconium-base alloy containing 1.5 at.% tin and small amounts of iron, chromium, nickel and oxygen, which has long been used to enclose fuel elements in water-moderated nuclear reactors) is embrittled by traces of metallic cadmium—solid, liquid, or dissolved in another liquid metal—in the narrow temperature range 300–340 °C. The fracture is purely brittle in character under these circumstances and takes place by cleavage on the (hexagonal) basal plane. The report is clearly preliminary and there is as yet no information about the time during which stress must be applied to generate fracture: it is a frequent characteristic of stress-corrosion that fracture is delayed.

This finding follows the discovery, ten years ago, that iodine can cause stress-corrosion failure in zircaloys (there are several variants of the alloy) in the range 250–500 °C. J. C. Wood (*J. Nucl. Mater.*, **45**, 105; 1972) studied the matter in detail for zircaloy held at 300 °C, which is a typical reactor-operating temperature for water reactors. For adequately large stresses and surface concentrations of iodine, fracture ensued, but previous anneals to remove residual internal stresses greatly reduced its incidence. Iodine-embrittlement is of peculiar concern because iodine, a volatile element, is a major fission product and is thus created inside the fuel element during service. Other fission products, however, would be expected to 'getter', that is, neutralise any free iodine; different metals can be regarded as being in competition for the attentions of free iodine. There was, in 1972, no clear evidence that any cracks in fuel element containers had been caused by this mechanism, and I have seen no later papers to suggest that any such events had been established. Wood's paper pointed the way to a number of precautionary measures.

Cadmium-embrittlement of zircaloy might conceivably be of consequence in a nuclear reactor because cadmium is one possible constituent of neutron-absorbing control rods, and Grubb's discovery may serve to discourage the use of this particular neutron absorber.

More generally, it is now clear that liquid metal embrittlement (if we may regard iodine as an honorary metal for present purposes) falls into two clearly distinct categories: hot or creep embrittlement, and cold embrittlement. It will be the next task of background research in this field to establish the characteristics of aggressor/victim combinations that predispose a system to one of these two modes of attack. □

Economics of aggression

from John Krebs

THE idea that cultural and genetic evolution are analogous is not new: both processes consist of the survival of a few out of many possible competing entities—ideas in the former case, genes in the latter. Some anthropologists and biologists have used this obvious parallel to develop the argument that human cultural traditions, like genes, have survived because they are adaptive (Harris, *Culture, People, Nature; An Introduction to General Anthropology*, Crowell, New York, 1971; Blurton-Jones, in *Growing Points in Ethology*, Cambridge University Press, 1976). The difficult question is to decide what is meant by adaptive. In the case of genes the answer is easy: successful genes survive because their individual owners are good at surviving and reproducing (Dawkins, *The Selfish Gene*, Oxford University Press, 1976), but do cultural traditions survive for the same reason? Most discussions by ecological anthropologists have emphasised group survival as a criterion of success of cultural traditions, which goes against the intuition of an evolutionary biologist well schooled in the view that differential survival of groups is not important in genetic evolution.

Recently W. H. Durham (*Q. Rev. Biol.*, **51**, 385; 1976) has discussed the cultural evolution of traditions of intergroup warfare in man, attempting to analyse the problem in terms of costs and benefits to individuals rather than groups. In animals, aggression is almost invariably associated with competition for resources such as food and mates, and whether or not fighting evolves as a means of competition depends on the costs and benefits to individuals (see below). Resources which are too widely scattered to be economically defendable are not usually exploited by aggressive competition. Durham suggests that this may be why Eskimoes living off migrating caribou and salmon have little tradition of intergroup hostilities, while those in South West Alaska living off more reliable sources of game do have traditions of intergroup warfare. In considering group conflicts, Durham argues that the payoffs to an individual will vary with the group size. A larger group will have more chance of winning, but there are more individuals to share out whatever resource is captured through the victory. Although the details may be complex, these trade-offs will lead to an intermediate optimum group size from the point of view of an individual in the group.

Durham discusses the example of the Mundurucú of Brazil in detail. These people traditionally lived in villages of about 200 individuals and engaged in intergroup warfare, collecting heads of rival villagers as trophies. The classical anthropological interpretation of this practice is that head hunting was a

Correction

In the report "Control systems in higher plants" (*News and Views*, **264**, 214; 1976) work of Daphne J. Osborne and coworkers was incorrectly described. The last sentence of paragraph 1, page 215 should read: "The rate of germination of normally slow (low vigour) embryos is however increased by a preliminary hydration, because during this period part of the early stage of germination is achieved. The biochemical changes are maintained when the embryo is dried again, so that when next supplied with water, such embryos complete germination more quickly, or are said to be 'enhanced'. In other words, if this treatment is given to whole seeds, a higher percentage will then emerge above the soil."

Planetary alignments don't cause earthquakes

from David W. Hughes

PLANETARY tides on the Sun influence solar activity, which in turn causes an increase in the solar cosmic ray flux reaching the Earth's upper atmosphere. This triggers unusual movements of large air masses, thus effecting the Earth's rate of rotation and subsequently triggering earthquakes. This, in a nutshell, is known as the 'Jupiter effect' mainly because Jupiter exerts the largest tidal influence on the Sun (this influence is proportional to Md^{-3} where M is the planetary mass and d its distance from the Sun, so that Mercury, Venus, Earth, Mars, Jupiter, Saturn, Uranus, Neptune and Pluto have relative tidal effects on the Sun in the ratios of 4.1 : 97.3 : 44.2 : 1.4 : 100 : 49 : 0.09 : 0.03 : 0.001) assuming the Jovian effect to be 100.) It was first proposed by K. D. Wood (*Nature*, **240**, 91; 1972) and popularised in *The Jupiter Effect* by John Gribbin and Stephen Plagemann (Macmillan London; Walker, New York; 1974) which generated considerable discussion and also a degree of criticism, counter argument and rejoinder (*Icarus*, **26**, 257, 268, 270; 1975).

Jean Meeus, the most severe critic, pointed out that according to this interpretation of the data, planetary alignments have an undetectable effect on sunspot activity In his opinion the effect doesn't exist. But Gribbin and Plagemann's argument is that planetary alignments are important and none more so than the 1982 superconjunction when Jupiter, Saturn, Uranus and Neptune will be colinear. These superconjunctions occur about every 178.73 yr. In this time Jupiter travels 15 times round its orbit plus an extra 24°, Saturn performs six revolutions plus 24°, Uranus two revolutions plus 46° and Neptune one revolution plus 30°. Meeus points out that a period of 178.73 yr should have no special significance as Saturn, Uranus and Neptune exert a rather minor tidal influence on the Sun in comparison with, say, Venus and Earth (see above). Tides raised on the Sun by Venus, Earth and Jupiter have a period of four months and this should be the dominant factor if any interrelation exists. The temporal similarity between the 11.1-yr cycle of solar activity and the 11.86-yr orbital period of Jupiter is thought to be entirely fortuitous. Also there are two tidal bulges produced on the Sun, one at the sub-planetary point and the other diametrically opposite, so the planets don't have to be all on the same side of the Sun as was asserted by Gribbin and Plagemann. The heliocentric opposition of January 16, 1901 should have been just as effective as the forthcoming superconjunction of 1982.

Despite these arguments Gribbin and Plagemann still "see no reason yet to discard the suggestion that planetary alignments do affect sunspots, let alone the more fundamental contention that solar activity affects the Earth's rotation and therefore earthquakes."

One way to solve this disagreement is to look at earthquake data to see whether the occurrence of earthquakes has a periodicity of about 179 yr. Gribbin and Plagemann peer into the future, predicting that 1982 is going to be a year of considerable activity and a possible date for the unwelcome movement of the San Andreas fault in California.

Also addressing this problem W.-H. Ip of the Department of Applied Physics and Information Science, University of California, San Diego, does without the crystal ball and takes a more orthodox scientific approach. In a recent issue of *Icarus* (**29**, 435; 1976) he describes how Yu Shen (in *Ke Yue Shi Yan* (*Scientific Experiments*), May 1975 page 22) has divided the period between 1000 AD and the present into quiet and disturbed periods of earthquake activity using ancient records of earthquakes in Northern China. Ip finds that since 780 BC only one of the 125 recorded earthquakes with intensities corresponding to 6 on the Richter scale coincided with a heliocentric planetary alignment, this one occurring in 1624 AD. Since 1000 AD none of the eleven earthquakes with intensities of 8 has coincided. These facts really do speak out against the Jupiter effect and Californians can, one hopes, rest more easily in their beds; San Andreas will undoubtedly move some time in the future but 1982 is no more likely to be the year than any other.

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cathartic activity promoting within-group harmony although, as Durham points out, it seems a rather extravagant method of promoting peace within the village. Instead, he suggests that the tradition is directly related to competition for a scarce resouce: game animals. The Mundurucú rely on game for high quality protein, and this is reflected in the importance of game in their religious rituals. Head hunting rituals also involved reference to game. A successful warrior was referred to as 'mother of the Peccary', referring to the fact that other village members were viewed as equivalent to game, and that obtaining a head trophy magically increased the fertility of game animals. This fits in well with Durham's interpretation that intergroup warfare is a form of individually selfish competition for game. More puzzling from the point of view of individual benefits, is the tradition that a successful warrior abstains from sexual intercourse for three rainy seasons. Not much good for individual fitness! Durham argues that the very scarcity of protein which precipitated the warfare would make procreation at that moment an unprofitable business (he doesn't say whether the whole tribe abstains), and that the warrior's payoff comes in other ways, such as exemption from future raids.

Durham's approach to interpreting cultural traditions of warfare is entertaining and makes intuitive common sense to a biologist, but whether it will really provide any predictive insight into differences between cultures rather than merely explaining after the fact, remains to be seen.

Returning to the costs and benefits of aggressive competition for food in animals, Carpenter and MacMillen, (*Science*, **194**, 639; 1976) have recently reported a quantitative study of territoriality in the Hawaiian Honeycreeper *Vestiaria coccinea*. Individuals of this species sometimes defend a feeding territory on flowering trees of the genus *Metrosideras*. Carpenter and MacMillen argue that territorial aggression will be economically advantageous as a means of securing food only if the gain in terms of increased nectar yield through excluding competitors exceeds the energy cost of territorial defence. Thus, for a given efficiency of excluding nectar thieves, territoriality will pay only if the average nectar productivity is above a certain level. (If productivity is low, no amount of exclusion of rivals will pay for itself.) At very high levels of productivity, there is so much nectar available that exclusion of rivals is not necessary, so honeycreepers should only defend food territories at intermediate levels of nectar production. By calculating the energy cost of maintenance for a territorial and non-territorial bird, as well as estimating the nectar production in each territory and the efficiency of exclusion of rivals, Carpenter and MacMillen could predict in a quantitative manner which birds should show territorial aggression, and which individuals should not. For nine out of ten birds (six territorial and four non-territorial) the behaviour was as predicted by the model. These results show how a simple cost-benefit theory can predict (as opposed to Durham's *post hoc* explanation) the occurrence of territorial competition for food in honeycreepers, although the authors suggest that an important additional factor is that the territorial birds defend more food than they need in the short term, perhaps as an insurance against hard times. ☐

Man's impact on nature

from Egon T. Degens

A Dahlem Conference on Global Chemical Cycles and their Alterations by Man was held in West Berlin on November 15–19, 1976. It was organised by Dr Silke Bernhard from whom further details may be obtained at Dahlem Konferenzen, D-1000 Berlin 33.

When man started to cut forest, burn steppe and plough land, the balanced system of air, water, life and soil was gradually disturbed. Through this type of land management, soil erosion rates quadrupled over the past 2,000 years and were borne with all their side effects by the environment, almost unnoticed by man. Industrial revolution and a growing population accelerated man's insult to nature to a point that the potential dangers are now inescapably apparent. Concerned people throughout the world are asking the now familiar questions: what is the present state of the global environment, where are we heading if increase in gross national product has higher priority than conservation, and what immediate steps are to be taken to improve the quality of the environment? At the most recent of the Dahlem Konferenzen, social and natural scientists addressed themselves again to some of these questions, though it was interesting to note that no member of the third world countries attended.

Some fourteen background papers (most of which, in my opinion, were rather catholic, indicating that we cannot yet give precise answers to global enquiries) elicited hundreds of written comments and questions. It was frustrating that only a few could be picked up in the general discussion.

Four groups addressed themselves respectively to the topics of models, modulations, and controls; fossil fuel burning and its effect on the biosphere and on sedimentary cycles; source functions: combustion products of fossil fuels, radionuclides and heat; and the resilience of the environment and of human society to changes in energy use. But it turned out that there is no way to deal reasonably with such an involved bundle of items, at least not during a five day session. Each group independently selected a few critical areas for discussion and almost identical key issues came up in the four huddles—man's impact on the global carbon, nitrogen, oxygen, phosphorous and sulphur cycles, and the interaction of C–N–O–P–S compounds with nature through these cycles.

Questions of immense importance turned out to have tremendously large uncertainties. One example may be taken—the question of whether global biomass is increasing or decreasing? Geochemists say it must increase, because they have a hard time finding another potential sink for the 'missing CO_2' in the atmosphere. This is so because, of the 4.6×10^9 tons of combusted carbon emitted every year as CO_2 into the air, only about 50% remains there; the rest apparently goes elsewhere, and it's not into the ocean. In contrast, biologists say the global biomass may remain steady or even decrease, partly as a result of excessive cutting of wood. They even believe that deforestation and the accompanying oxidation of wood and humus is a significant contributor of CO_2 to the air, and values between 1×10^9 and 5×10^9 tons of C could be annually released to the air if one uses some of the published figures

So it seems that the increase in atmospheric CO_2 is not solely due to the burning of fossil fuel but that deforestation is also an important pollutant. How can we reverse this trend? Should we plant a trillion trees, as somebody suggested? But to be realistic, there is no way out than to cut CO_2 emission to the atmosphere over the next 20 years or man will be warm but very, very sorry. Although the climate curve shows a cooling trend at present, only a minority of one-and-a-half was of the opinion that the impact of man-made CO_2 on climate is beneficial. As well as producing CO_2, fire and fossil fuel burning also release particles to the air. How do these affect global climate? Does stratospheric light-scattering by particles lower world temperatures or are the particles more efficient absorbers than scatterers and thus raise the global temperature? No opinion one way or the other was arrived at.

Equally uncertain were questions of the significance of man's perturbation of the ozone layer compared with natural perturbations. What are the quantitative effects of aerosols, N_2O, or CO? It turned out that the data base for ozone is not good. There are not only chemical but meteorological changes and not all participants shared the view that man causes the alterations noticed in the ozone layer. Are we kidding ourselves, because we ask questions on these and related issues that we cannot answer? The whole reputation of science is at stake, because in the past so much has been made of spray cans, fuel combustion and other environmental hazards caused by man's activities. The scientist's role is to bring about a change in technological approaches, which may lighten the burden on nature and at the same time help to fulfill the social needs of all the people in the world, and not just those from the developed countries. To accomplish this the scientist needs to put forward an exceptionally credible case, because the biggest inhibition of public action is disagreement among scientists.

In conclusion one of the finest results of the Dahlem Konferenz was that all participants tried to agree on the range of uncertainties, rather than being disappointed at not coming to a scientific consensus. There is a long way to go before global models of chemical cycles are universally accepted. At present the question of man's impact on sources and sinks of the biogenic elements is still up in the air.

Why marsupials can't win

from Barry Cox

One of the most intriguing problems in vertebrate evolution is the reason for the superiority of eutherians ('placentals') when competing with marsupials. Because they are so alike in their levels of morphological adaptation, it has long seemed likely that the cause lay within the fundamentally different patterns of reproductive physiology of the two groups. In an extremely interesting paper, Lillegraven (*Evolution*, **29**, 707; 1976) has recently examined this possibility.

Lillegraven commences by reviewing a number of papers by Müller, whose contributions have been largely ignored (unread?) by English-speaking workers. Marsupials are born nearly helpless, at a very early stage of development, since they have a very short period of gestation. Müller has shown that their pouch young show several features adapted to life in the pouch, such as seals to close the lips around the nipple, to protect the eyes and to plug the external auditory meatus. All these adaptations occur fleetingly in the development of eutherians, strongly suggesting that they, too, originally had a developmental history like that of living marsupials.

Although Müller herself regards the marsupials and eutherians as having developed their reproductive strategies independently from an oviparous common ancestor, Lillegraven rejects her reasons for this conclusion. His own earlier work (*Palaeont. Contribs Univ. Kansas*, **50**, 1; 1969) has shown that a comparison between the details of the structure and development of the membranes and early embryos of a didelphid marsupial and a eutherian show no features that exclude the marsupial system as phylogenetically ancestral to the eutherian system. Even the marsupial pouch is absent in such forms as the didelphid *Marsoma*, whose young swing freely from the nipples, and Lillegraven suggests that such a developmental history was present in the common ancestor of marsupials and eutherians. Such a relationship is also supported by modern palaeontological work that Müller had not considered.

Most marsupials have a gestation period of only 11–15 d. Though in some this is extended up to 38 d, this extension is not accompanied by a corresponding advance in the stage of anatomical development that has been reached at birth; instead it is accompanied by a slow-down or standstill in development. It seems as though there is a natural limitation to the amount of growth and development that can take place within the marsupial uterus, so that beyond this point the embryo must be transferred to the extrauterine nipple.

Lillegraven suggests that this limitation is imposed by the natural incompatibility between the 'foreign' foetal tissues and the tissues of the mother. Intimate relationships between these two tissues would trigger the highly efficient immune response, leading to immunological rejection by the maternal haematopoietic system. Though a placenta does develop in some marsupials, this problem restricts the duration and intimacy of relationships between foetal and maternal tissues. So the marsupial embryo can develop within the uterus only until its own food reserves in the yolk and abdomen are exhausted, and then must be born before the mother can mount a fully-fledged immunological attack.

The ability of eutherians to avoid this immunological crisis is due to the evolution of the trophoblast, which provides the major barrier between the foetal antigens and the maternal antibodies. At least one trophoblast layer always lies between foetal and maternal tissues within the placenta. The mechanism of inhibition of the maternal immune response is still uncertain, and seems to include physical separation by non-cellular trophoblasts, the development of tolerance within the mother due to transfer of materials from the placenta, and the active involvement of the trophoblast in opposing rejection of the foetus. These adaptations made it possible for the placenta to be used for respiratory and excretory exchange and for nutrition of a yolk-less embryo over a prolonged period of time. The trophoblast also transmits maternal immunoglobulins to the foetus, and is responsible for synthesis of hormones allowing prolongation of pregnancy. As a result of all these adaptations, the eutherian young can be born at an advanced stage of development, and already possessing immunity to some infections.

As Lillegraven points out, the marsupial type of development also limits that group to producing a neonate that can independently reach the nipple and survive there breathing air. This prohibits the evolution of fully aquatic marsupials, or of marsupials in which the forefeet are specialised into flippers, non-opposable fingers or single-toed hoofs. (This perhaps explains why the Australian answer to the eutherian ungulates is the kangaroo.)

At least, with living marsupials to examine and compare with their eutherian relatives, it is possible to identify such a biochemical reason for their competitive inferiority. If marsupials were extinct, palaeontologists today would be faced with an insoluble problem! ☐

Ligand binding properties of the haem group

from A. J. Thomson

DEOXYMYOGLOBIN and deoxyhaemoglobin contain the paramagnetic form of the haem group as a result of the presence of the high spin ferrous ion. It is now accepted that in this spin and oxidation state the iron sits out of the plane of the haem since it is too large to fit into the hole in the ring. On binding oxygen these proteins become diamagnetic and the iron atom drops back in the centre of the haem ring. It has been proposed, in the case of haemoglobin, that, on binding oxygen, this movement of the iron atom triggers a change in the quaternary structure of the protein which leads, in turn, to an increase in the oxygen affinity of the remaining haem residues in the protein. This mechanism is thought to be the molecular basis for the Bohr effect (Perutz, *Nature*, **228**, 726; 1970).

The nature of the oxygen–iron binding has been controversial in two respects, however. First, the stereochemistry of the iron–oxygen unit has, until recently, been much debated in the absence of good structural data. Second, the formal oxidation state of the iron atom has been in dispute.

From X-ray studies of the synthetic model compounds 'picket-fence' porphyrins ($\alpha,\alpha,\alpha,\alpha$-tetrapivalaminophenylporphyrin, TpivPP) which bind oxygen reversibly, it has been determined that the dioxygen binds iron in a bent end-on fashion with a Fe-O-O angle of 130° (Collman *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1326; 1974). But the formal oxidation state of the iron has proved more difficult to resolve.

The problem was to distinguish between two possible states. Pauling (*Nature*, **203**, 182; 1964) has described the system as low spin ferrous iron bound to dioxygen which has become spin paired. This presumably implies little transfer of electronic charge from the iron to the dioxygen ligand. The second view first put forward by J. J. Weiss (*Nature*, **202**, 83; 1964) suggested that one electron is transferred to give a species Fe^{III}–O_2^-, the low spin ferric ion and a superoxide anion, with their electron spins anti-ferromagnetically coupled to give a diamagnetic resultant.

To probe the nature of the bound oxygen ligand and to distinguish between a superoxide ion and a neutral dioxygen the infrared stretching frequencies have been measured. This frequency drops steadily as negative charge is added (for example O_2, 1,556 cm^{-1}; O_2^-, 1,150–1,100 cm^{-1}; O_2^{2-}, 740–850 cm^{-1}). Caughey and his coworkers (*Biochem. biophys. Res. Commun.*, **55**, 91; 1973; *Biochem. biophys. Res. Commun.*, **58**, 166; 1974) in pioneering experiments succeeded in measuring the dioxygen stretching frequencies in both oxymyoglobin and haemoglobin, obtaining values of 1,103 and 1,107 cm^{-1} respectively. On substitution with the isotopically-labelled species $^{18}O_2$ shifts to 1,065 cm^{-1} were seen in both cases, adequately confirming the assignment of the bands. Although these values clearly lie in the superoxide range Caughey and his collaborators do not favour a formulation as ferric superoxide, rather they prefer to talk of strong covalent bonding between the iron atom and dioxygen. Initial attempts to measure the oxygen stretching frequency in the model Fe-TpivPP compounds produced a value of 1,385 cm^{-1} (Collman *et al.*, *J. Am. chem. Soc.*, **96**, 6522; 1974). It is now known, however, that this result was an artefact caused by an unknown impurity in a KBr disk. Recent measurements place the value at 1,163 cm^{-1} in a Fe(TpivPP)(NTrIm)O_2 model and 1,159 cm^{-1} in Fe(TpivPP)(NMeIm)O_2, where NTrIm is 1-tritylimidazole and NMeIm is 1-methylimidazole. Isotope shifts on substitution with $^{18}O_2$ are used to confirm assignments (Collman *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 3333;

1976). This revision brings the model compound values close to the haemoprotein values, all now lying within the superoxide range.

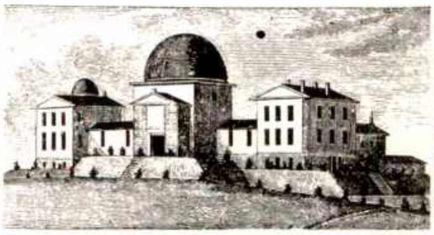
A comparison can usefully be made with a range of cobalt–porphyrin derivatives which also bind oxygen reversibly. Caughey and Maxwell (*Biochem. biophys. Res. Commun.*, **60,** 1309; 1974) have shown that the dioxygen stretching frequency in the cobalt(II)porphyrin analogue of haemoglobin is 1,105 cm^{-1}. Now the recent report from Collman, *et al.*, puts values of 1,153 and 1,150 cm^{-1} to the cobalt 'picket-fence' analogues with different axial bases. The change of the metal ion has not significantly altered the stretching frequency. But since the cobalt analogues possess one more d-electron than the iron compounds an EPR signal can be obtained. It was shown by Hoffman and Petering (*Proc. natn. Acad. Sci. U.S.A.*, **67,** 637; 1970) that the cobalt–oxygen adduct gives a signal with no cobalt nuclear hyperfine structure visible. They therefore concluded that the odd electron is largely on the dioxygen and that the formulation Co(III)–O_2^- is the most appropriate. In view of the similarities between the stretching frequencies of the dioxygen when bound to cobalt or iron model porphyrins and haemoproteins it is difficult to resist the conclusion that J. J. Weiss was correct in 1964.

In their oxygen-binding properties the picket fence porphyins model the haemoproteins well. An interesting difference arises, however, in their very different affinities for the ligand carbon monoxide. The picket fence model binds CO irreversibly whereas the oxygen-carrying haemoproteins have considerably lower binding affinities which vary in the different mutant forms, in other words, as a function of protein structure. Collman *et al.* have now pointed out that the CO stretching frequency drops as the CO affinity of the haem group drops. It is suggested that the CO affinity in the protein is lowered as a result of the steric constraints of the haem pocket's causing the Fe–CO group to become bent and the iron–carbonyl bond to be weakened. Because the Fe–O–O structure is intrinsically bent its affinity is not correspondingly reduced. This ability to discriminate against CO binding may be of crucial importance since the biological catabolism of haemoproteins is a primary endogenous source of CO. The authors conclude that poisoning by CO of both haemoglobin and myoglobin would be considerably greater if the CO ligand were not forced to tilt.

It is admitted, however, that it may be an oversimplification to ascribe all the differences between CO and O_2 binding to the steric constraints of the haem pocket. In planar systems with trans-axial ligands there is an appreciable electronic affect transmitted across the metal plane. This was nicely shown in model studies using cyano-cobalamin, which contains the cobalt(III)-corrin ring system. The infrared stretching frequency of the bound CN$^-$ ligand was measured as a function of the ligand *trans* to it (Firth *et al.*, *J. Chem. Soc. A*, 2428; 1968). There is a perfect correlation between the binding constant of the cyanide group and its infrared frequency; the frequency drops as the binding constant drops. The decrease in affinity for cyanide is brought about by a change in the *trans*-axial ligand and can only be due to an electronic effect transmitted by way of the metal ion. Now it is known that in the haem models Fe(deuteroporphyrin) (2Me-Im) the CO is bound 200 times less well than in Fe (deuteroporphyrin) (Im). 2-methylimidazole (2Me-Im) is a sterically hindered base which cannot approach the porphyrin plane closely (Rougee and Brault, *Biochemistry*, **14,** 4100; 1975).

Thus there is also a pronounced electronic *trans* effect in the iron–porphyrin system. Another function of the protein in the oxygen-carrying proteins could well be to control the distance between the haem plane and the bound histidine residue, thereby affecting affinities for *trans* ligands. It is not yet known whether such control will affect the affinities for both CO and oxygen in a parallel manner. ☐

A hundred years ago



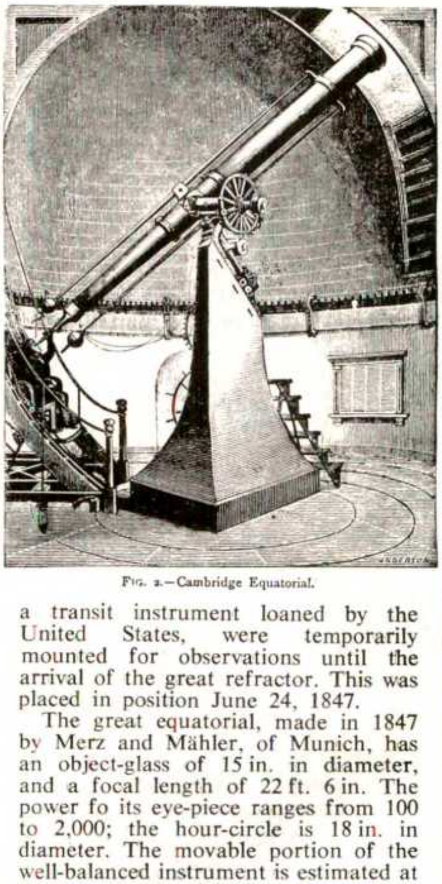


Fig. 2.—Cambridge Equatorial.

CAMBRIDGE (U.S.) OBERVATORY

A look into the earlier annals of the observatory of Harvard repays the inquirer at the outset by revealing the interest in astronomical pursuits which was felt in the old Bay State many years before the founding of an observatory was practicable in the United States. In 1761 the *Province* sloop was fitted out at the public expense to convey a Harvard professor, Winthrop, to Newfoundland, to observe the transit of Venus of that year; and in the troublous times of 1780 the old "Board of War" fitted out the *Lincoln* galley to convey Prof. Williams and a party of students to Penobscot to observe a solar eclipse. At so early a day was New England disposed to encourage scientific observations.

A new issue now arose. The sudden appearance of the splendid comet of 1843 was, happily, the occasion of final success in the founding of the present institution. Cambridge was immediately appealed to for information about this strange comet. But the observers had no parallactic instruments or micrometers of the least value for its observation. While they were endeavouring to obtain data to compute the comet's orbit, a meeting of citizens was held, under the sanction of the American Academy, to take measures for procuring a first-class equatorial; the needed amount of $20,000 for the instrument was contributed in Boston, Salem, New Bedford, and Nantucket. The equatorial was ordered from Merz and Mähler, of Munich, and Harvard determined to erect a new observatory. The location selected was 80 ft. above tide-water, and 50 ft. above the plain where the soil was found favourable for the stability of piers for the instruments. In 1844 the buildings were occupied, and an equatorial of 44 in. focal length and 2½ in. aperture, and a transit instrument loaned by the United States, were temporarily mounted for observations until the arrival of the great refractor. This was placed in position June 24, 1847.

The great equatorial, made in 1847 by Merz and Mähler, of Munich, has an object-glass of 15 in. in diameter, and a focal length of 22 ft. 6 in. The power fo its eye-piece ranges from 100 to 2,000; the hour-circle is 18 in. in diameter. The movable portion of the well-balanced instrument is estimated at three tons. Its original cost was about $20,000. The sidereal motion given to this telescope is now secured by clock-work from Alvan Clark, which is spoken of by the observers as the only known "driving-clock working with perfect steadiness." The telescope rests on a central granite pier, in constructing which 500 tons of granite were used. It is 40 ft. high, and rests on a wide foundation of grouting 26 ft. below the ground surface. Upon the top of the pier is laid a circular cap-stone 10 ft. in diameter, on which is the granite block, 10 ft. high, bearing the metallic bed-plate. This instrument is in the central "Sears Tower."

from *Nature*, **15,** January 4, 201; 1877.

articles

Stonehenge I and lunar dynamics

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The concept of Stonehenge I as an accurate predictor of eclipses can be criticised on the grounds of inadequate short-term precision, unnecessarily good long-term tracking of the lunar nodes, and irrelevance to cultural needs. A more practical application of the astronomical characteristics of the structure is that of lunar apogee tracking, which would allow prediction of minimum tidal disturbance to cultural communication across the North Sea and English Channel.

FOLLOWING Hawkins[1] numerical analyses and discussion of the alignments between components of the Stonehenge monument on Salisbury Plain, England, (described by Atkinson[2]) the succeeding decade of investigation has further emphasised the dichotomy of viewpoints between the astronomers and the archaeologists. Some of the original deductions of Hawkins have been strongly criticised by both groups[3–6], but the work of Hoyle[7] and of Newham[8] has extended other aspects and shown that Stonehenge I, the earliest phase of the megalith, could have been used as a universal eclipse predictor, independently of any eclipse cycle.

Operation of the device in this mode would have been simple, but the sophisticated intellectual reasoning needed to design the system conflicts to a high degree with the otherwise satisfactory archaeological picture of Britain as having a technologically primitive agricultural economy. Although now endorsing an astronomy-oriented content in the Stonehenge structure, most archaeologists resile from the 'eclipse computer' concept *per se*, because of this incompatibility, preferring religious connotations to explain the interest in the Sun and Moon[6].

Stonehenge I

The concensus of opinion is that the initial phase of Stonehenge consisted of:

(1) The raised bank and quarry/ditch (both originally about 2 m from datum).

(2) The circle of 56 pits, named 'Aubrey holes' (A holes), filled in shortly after their original excavation. Erosion of the surface since the construction date makes their original form doubtful, but it is noteworthy that the excavated material, replaced in the hole, would exceed the pit volume and form a chalk mound, an ideal quasi-permanent marker of high visibility in twilight conditions. The name Aubrey holes may be rather misleading.

(3) The primary alignment, represented by a line from the central point to the heelstone[9], with its associated quadruplet of equally spaced 'A' postholes. This is not the axis, which is of phase II/III origin.

(4) The 'causeway' postholes at the 'entrance'[8].

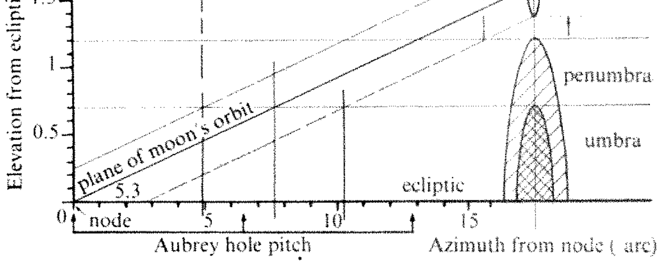
(5) The *locations* of the 'station stones'. The actual station stones appear to have phase III characteristics, but the locations are deemed significant, and relevant to phase I because of their near-rectangular geometry resulting from the choice of latitude for the Stonehenge site[8].

Angular resolution and the Aubrey holes

It is a weak point in the astronomical argument that, as an eclipse predictor, Stonehenge I is embarrassingly comprehensive, potentially capable of predicting all eclipses, when only about half could be observed from a single location. A much more stringent criticism, however, is the inadequacy of the moment-to-moment accuracy of the tracker markers in the 56-step 'plan position indicator' scheme of Hoyle. An unsynchronised incremental movement of 6.43° in each of three markers, Sun, Moon, and node-pair, gives a potential average angular error of $\sqrt{3}$ Aubrey holes, or more than 11° arc. The Lunar eclipse prediction requirement can be assessed from Fig. 1 and it can be seen that an 11° error would be sufficient completely to miss even an umbral eclipse if it were tangential. Moreover, solar eclipses require better than twice the resolution needed for lunar eclipse prediction. At any time in the operation of Stonehenge I, the average error could have been halved by a simple doubling of the number of Aubrey holes and of the rates of marker movement, so it is reasonable to suspect that eclipse prediction was not the prime purpose of the Stonehenge I Aubrey hole array.

The number 56 of Aubrey holes is of critical importance in the circumstantial evidence for the astronomical case. Fifty-six is very close to three times the nodal regression period in years (55.835), and has led most investigators to accept the relevance of the extraordinary accuracy of node tracking implicit in this close match. Node markers moved around the Aubrey circle at three Aubrey holes per year (A.H. yr^{-1} hereafter) relative to a fixed 'equinox', accumulate an error of only one Aubrey hole in more than a century, and can thus easily be reset by 93 yr periodic

Fig. 1 The angular relationships between mean lunar angular diameter and the Earth's umbra and penumbra, in a lunar eclipse situation. The incremental angular resolution of the Aubrey hole array would not be satisfactory for a definitive prediction of an eclipse, if the average error were $\sqrt{3}$ Aubrey holes (see text).



recalibration observations of maximum northerly midwinter full moonrise azimuth. The figure of 93 yr is arrived at because five times the nodal regression period is 93.06 yr, adequately close to an integral number of midwinter solstices.

The logic of this interpretation of the significance of the number 56, however, is suspect. The argument of Hoyle is that the most northerly azimuth of midwinter fullmoonrise (the peak of the nodal cycle), was determined by interpolation, using the A posts (and thus achieving independence from a rather infrequent coincidence of fullmoonrise with winter solstice) primarily for the purpose of determining the date of that event, the reset moment of the nodes markers. But it is a simple fact that the cancellation of any accumulated error in the nodes markers could have been achieved many times per decade, by observation of lunar eclipses. Any total lunar eclipse of long duration would automatically define the near-zero angular displacement of the nodes from the Sun–Earth–full moon axis to a precision rather superior to that achievable by deducing the date of maximum northerly virtual fullmoonrise (abbreviated hereafter to MNVF).

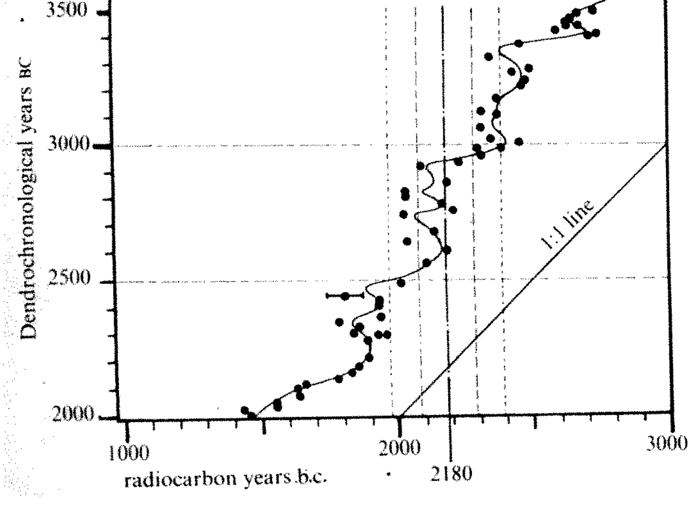
The concept of the interpolation technique is valid, provides an elegant solution to the use of the A postholes, and is highly relevant to the argument that follows, but the task assigned to it by Hoyle cannot be considered logically justified.

The new dating

The radiocarbon dating revolution within archaeology has caused a considerable reassessment of the process of cultural diffusion from Western Asia to Europe, and of the relation of Stonehenge to the Wessex culture. Following the publication by Suess[10] of his dendrochronologically-based calibration chart for radiocarbon samples, Stonehenge I in particular has become very difficult to assess. The most relevant sample, I-2328, chosen as representing the earliest construction phase, is from the antler pick found 3 in above the bottom of the outer ditch at 6 ft depth, in the primary silt[9]. Isotopes Inc. dated this at 2180±105 b.c.

The present position is summarised in Fig. 2. Because of the 'staircase' curve, the new calibration would give essentially the same radiocarbon date for samples of real origin dates between about 2500 BC and 3000 BC. (The reality of the Suess calibration curve retrogressions has of course been questioned, but the larger ones, such as those in Fig. 2, do seem to be caused by an actual fluctuation in the ^{14}C levels of the period[11,12].) Further sample dating is urgently needed.

Fig. 2 The Suess dendrochronologically-based radiocarbon calibration curve for the calendrical interval 2000–3500 BC (adapted from ref. 10), with the radiocarbon age data for Stonehenge I sample I-2328 (2180±105 BC) superimposed. The vertical dashed lines define the normal distribution confidence levels of 68% and 95%. Suess' typical dating uncertainty limits are given for one of his points; the limits for the other points have been omitted for clarity.



The anomaly of Stonehenge I is now better realised. The uncertainty of dating does not obscure the probability that this geometrically established structure predates the main building phase of the Egyptian pyramids, and that it may have been contemporary with the proto-literate Sumerians[13].

An important outcome of the new dating is the modification now necessary to the values of parameters used to calculate the positions of some astronomical entities. It will be seen that the date regime 3000–2500 BC is of great significance to an improved understanding of Stonehenge I.

Static geometry and dynamical astronomy

In the past the emphasis has been on the midsummer sunrise phenomena at Stonehenge. For this to have obscured for so long the much better match of the structural geometry to the midwinter moonrise phenomena, is rather surprising. The entrance includes the range of moonrise azimuths from that represented by the heelstone, to the MNVF azimuth, and is asymmetric with respect to the avenue. This same range of azimuths covers the causeway postholes, investigated by Newham, all of which are northward of the heelstone azimuth and which have three ranks terminating close to the MNVF azimuth.

This emphasis on the extreme northerly azimuth of moonrise is evidently very important to investigations of the motivation behind Stonehenge I, and now that a more definitive range of radiocarbon dates has been derived for this part of the structure, it is relevant to attempt the determination of the actual solsticial moonrise azimuths for that period, so as to achieve a greater understanding of the designer's motivation.

I have used the polynomial expressions for the angular positions of features of the lunar orbit, given in the explanatory supplement to *The Astronomical Ephemeris* (HMSO, annually), to examine the moonrise azimuth situation in the period 3500–2000 BC. When using these expressions it must be remembered that the imprecisely known secular acceleration of the Moon leads to some uncertainty regarding the lunar longitude at times remote from the AD 1900 epoch, but it is considered that the expressions for the lunar node and perigee are accurate to a few degrees for periods up to 100 Julian centuries (Her Majesty's Nautical Almanac Office, personal communication). The parameter T (Julian centuries) is common to all the polynomial expressions, and its value for each winter solstice of the relevant years can be determined by solving for T the solar longitude expression when the longitude is defined at 270°. The accurate value for T can then be inserted into the expressions for obliquity of the ecliptic, and for the longitudes of the Moon's ascending node and perigee.

The spherical trigonometry used to determine from these parameters the azimuth of virtual moonrise has been described elsewhere[7] (moonrise is defined as the tangency of the Moon's lower limb to the horizon). Assuming the present-day value for the eccentricity of the lunar orbit, and that the atmospheric conditions were 1002 mbar and 10 °C, as used by Hawkins[1], then with the measured value of 0.60° for the elevation of the relevant portion of the Stonehenge horizon, the virtual moonrise azimuth can be determined to a high accuracy (typical uncertainty for ± 50 mbar in atmospheric pressure, for example, would be ± 0.05° arc in azimuth).

Coincidence of azimuths

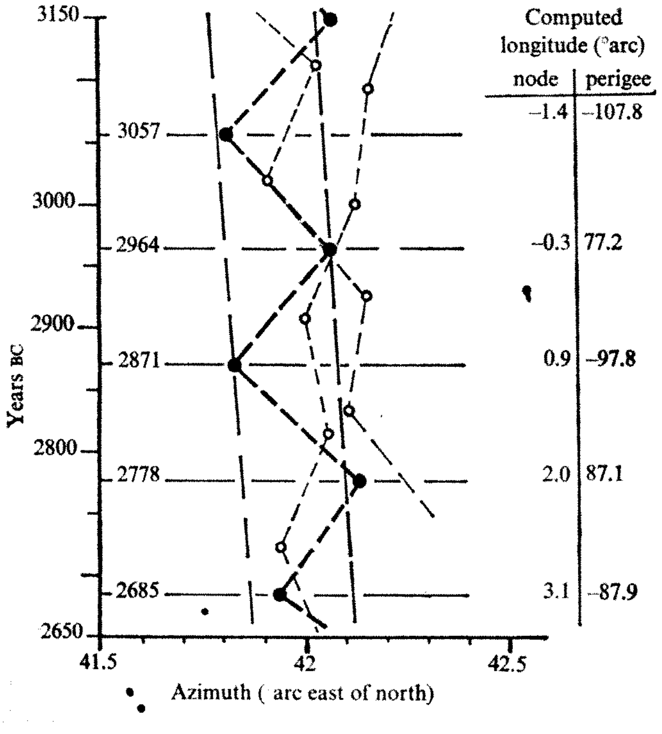
The conditions for extreme northerly virtual fullmoonrise are that the line of nodes be exactly in quadrature at the winter solstice (the longitude of the ascending node being zero), and that apogee be at the virtual fullmoon point, for minimum horizontal parallax. The first criterion alone is a moderately rare phenomenon, even when allowing a few degrees of longitude tolerance. Within the arbitrary limits of ± 1 A.H. of longitude, eligible coincidences occur approximately three times every 93 yr. Because it is at this time in Hoyle's scheme, that the nodes marker-pair is checked for 'longitude' accuracy, however, it is highly significant that the normal incremental angular resolution of 1 A.H. is much improved by a knowledge of the temporal mismatch between nodal quadrature and winter solstice.

For the second criterion to coincide with the first is rather more rare. To an arbitrary limit of 2 Aubrey holes of longitude accuracy (for a hypothetical apogee marker), coincidence occurs at intervals of about 1247 yr (67 cycles of nodal regression and 141 cycles of apogee precession).

A computer programmed to perform the necessary search, showed that at the winter solstice of 2871 BC ($T = -47.699$ Julian centuries from AD 1900), the ascending node of the lunar orbit had a longitude of about 1°, and apogee was about 7.8° from the virtual full moon point. Figure 3 shows the sequence of MNVF azimuths between 3250 BC and 2400 BC. Of particular interest is the sequence of apogee MNVF azimuths for the years 3057, 2871 and 2685 BC. At 41.8°, 41.83° and 41.88°, they are very close to the extreme limiting azimuths possible at those times.

It is remarkable that the causeway postholes investigated by Newham[8] contain three ranks terminating at azimuths which he measured at 41.8°, 41.9° and 41.9°.

Fig. 3 Computed azimuths of virtual fullmoonrisings at winter solstice, in the calendar date regime 2650 BC to 3150 BC, as seen on the NE horizon of Stonehenge, when the ascending node of the lunar orbit was less than one Aubrey hole pitch from the equinox. The pair of nearly vertical dashed lines are the azimuth limits for apogee and perigee when the longitude of the ascending node is zero. The rare sequence of nearly perfect apogee and perigee azimuths, which occurred in the Stonehenge I period, is shown emphasised.



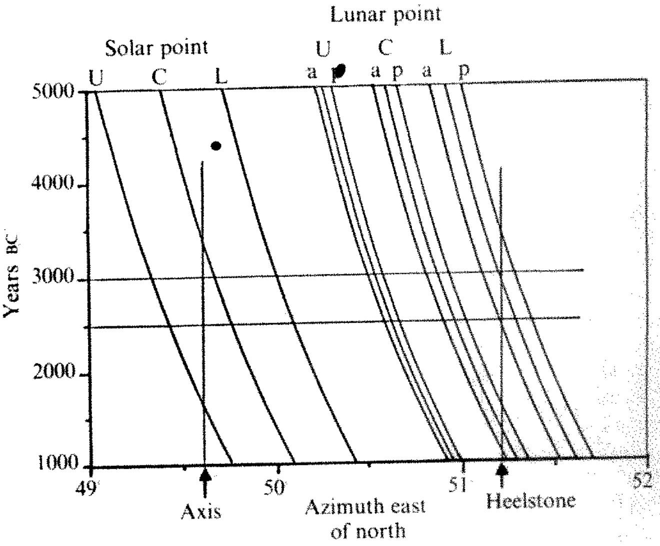


Fig. 4 The heelstone azimuth. The curves show the rising azimuths for the upper, (U), and lower, (L), limbs, and for the geometrical centre, (C), at winter solstice and nodal syzygy for the Moon, and at summer solstice for the Sun. Apogee and perigee displacements are given for the Moon. The general shift of all azimuths with time is due to the changing obliquity of the ecliptic. The 2500 and 3000 BC time lines facilitate correlation with Fig. 2. The axis azimuth is approximate only, but the heelstone azimuth was accurately measured by Hawkins[1], as 51.222.° At the winter solstice of the year 2857 BC, the ascending node of the lunar orbit had a computed longitutude of 90.0° (syzygy) and the azimuth of fullmoonrise for the lower point was computed to be 51.28°.

Such a close coincidence encouraged me to pursue the investigation and examine the remaining feature of Stonehenge I at the other extreme of the entrance azimuths, the heelstone. Robinson[9] had pointed out that the heelstone was a better marker of the node at winter solsticial syzygy than it was of midsummer sunrise, and my computations bear this out to a remarkable degree. Figure 4 demonstrates the striking match of the heelstone azimuth to that of the moonrise azimuth for nodal syzygy in the date regime centred on 3000 BC (again for lower limb tangent to the horizon).

For the sinusoidal function of winter solsticial moonrise azimuth to be marked in the Stonehenge I structure at no less than four points (the rare triple extreme event and the 'crossover' point) is prime evidence for a knowledge of the existence and of the accurate position of the lunar node, that can be described only as astonishing in its precision.

Moreover, the marking of the apogee MNVF azimuths and the omission of the perigee equivalents has some interesting connotations.

Apogee tracking

In Hoyle's scheme, the most accurate tracking is that of the nodes, which in 2871 BC accumulated an error of 1 A.H. every 111 yr (when moved at 3 A.H. yr^{-1} relative to a fixed 'equinox'). This figure is arrived at by differentiating the polynomial expression for the longitude of the lunar node, with respect to time, and inserting the appropriate value of T into the resulting expression, to obtain the real regression rate (18.6107 tropical years per rev.) and then taking the reciprocal of the difference between the ideal Aubrey hole marker rate (3.00902 A.H. yr^{-1}) and the prescribed rate.

Remarkable though this accuracy (or coincidence) seems, it is outweighed by an even more accurate relationship. Performing the same calculation for an apogee marker, the precession period is found to be 8.84516

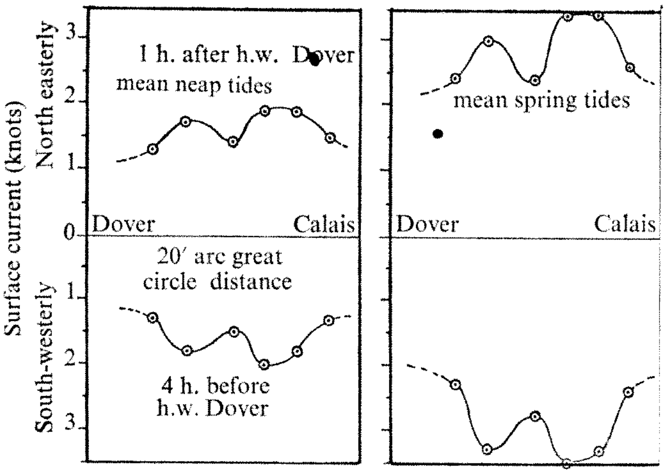


Fig. 5 Present-day surface currents in the Dover Strait. By rowboat, at 2.5 knots, the 20′ arc surface traverse would require 8 h if there were no tidal disturbance. For a daylight journey, from embarkation to landfall, the most suitable tidal conditions would be those at apogee neaps, when the peak surface current may be expected to be about 13% less than than at mean neaps.

tropical years, the ideal A.H. tracking rate 6.33115 yr^{-1}, and the error accumulation rate, for a marker moved at 6.33333 A.H. yr^{-1} (6, 6, and 7 Aubrey holes in a repeating triennial sequence), 1 Aubrey hole every 458 yr. Only for 56 Aubrey holes does this combined accuracy exist for both nodes and apsides trackers.

Such circumstantial evidence has been the basis for all previous astronomical interpretations of Stonehenge, especially the eclipse predictor aspect mentioned above, but it is also true that the presumed cultural motivation for Stonehenge I has been specified to match these hypotheses. Since eclipses could have served no practical purpose in what was essentially a subsistence farming economy, the motivation has been stated as 'religious'; surely the lowest common denominator of imprecision and evasion.

The hypothesis discussed below is an attempt to demonstrate a strong cultural motivation for the origin of Stonehenge I, in which the observations of natural phenomena are logically extended, and lead to the analogue device which has for so long puzzled a multitude of investigators. The only assumption that need be made is that the spectrum of intelligence present in the indigenous population of Neolithic Britain and Europe, was essentially the same as it is today, surely an acceptable criterion in view of the precision of azimuthal delimiting described above.

The relevance of tides

Thom[14,15] has suggested that the background to the north British megaliths could have been an interest in the lunar influence of tides, but these structures he dated from astronomical considerations, to about 1750 BC. Nevertheless, there may be a connection with the 1,000 yr older Stonehenge I lunar observatory.

The pre-Wessex south British cultures seem to have been culturally isolated from mainland Europe during the Stonehenge I period. A major factor contributing to this situation may have been the difficulty of traversing the strongly tidal English Channel, Dover Straits, and North Sea. To the operator of any surface craft whose speed is limited by the use of sails or oars, the need for tidal prediction is obvious, even today. In 2750 BC with sea levels averaging 4 m lower than at present[16], and tidal currents over the continental shelf therefore appreciably more severe, and with the surface craft operational qualities perhaps less adequate than those of modern craft, the situation could have been even more relevant.

Figure 5 has been derived from a standard tidal stream atlas[17], and shows the present-day strength of the tidal streams in the Dover Strait. The high values of stream velocity over the entire width of the Strait emphasise the need for small craft to choose conditions of minimum tidal disturbance for a safe and reliable crossing.

Consideration of the criteria determining tidal phenomena shows that the maximum tide-raising force exists when the following priority-ordered conditions are met simultaneously: (1) Lunar phase 'New' or 'Full', with an eclipse as the extreme event; (2) Moon at perigee; (3) Earth at perihelion (2 and 3 because of the inverse-cube law of tide-raising force with separation distance); (4) Earth at equinox, for peak semi-diurnal tide; (5) Line of nodes in syzygy. Condition (1) is virtually the defining parameter of 'spring tide', with (2) as a major modulator of the effect, having a force ratio, perigee–apogee, of 1.39. The force-ratio for (3) is 1.126. Condition (4) is important since the Atlantic Ocean responds mainly to the semi-diurnal tide-raising force[18]. Least important is (5), serving only to reduce to zero the very small effect of a few degrees separation of the solar and lunar radius vectors.

With the lunar perigee–apogee situation known to have existed at Stonehenge in the period 3057–2685 BC, Neolithic observers had a significant opportunity to notice that the difference in spring tide range correlated with the observed change in MNVF azimuth, and thus to link these two phenomena. But for this to be fortuitous is of far too low a probability to be taken seriously. Some link between the variation in tide range and the lunar motion, must have been suspected for the motivation to be established to carry out detailed observations as evidently occurred at Stonehenge.

The first order effect of condition 1 is obvious enough, but the modulation due to (2) might seem rather more difficult to observe. There is however, a curious near-commensurability in the relevant intervals which immensely aids observation. Expressed in terms of the lunar motion:

Synodic month (phase to phase):

$$29.53089\ \text{d}\times 14=413.428\ \text{d}$$

Anomalistic month (perigee to perigee):

$$27.554551\ \text{d}\times 15=413.318\ \text{d}$$

$$\text{Difference}=0.11\ \text{d}$$

Hence the perigee/apogee axis moves through one lunar phase cycle in slightly less than a 14-lunation period. In terms of the tide-raising force, therefore, there is evidently a periodic modulation in the range of the Spring tide, and of the strength of the tidal currents, occurring cyclically over 14 lunations, with maxima at 7-lunation intervals, alternately at New and Full Moon. Similarly the neap tide effects vary with the same periodicity, with minima at every seventh first and third quarters.

If this 14-lunation periodic effect had been exact, then possibly no problem would have existed, but the slow phase shift between the incommensurable periods of lunar phase change and perigee precession would destroy the relationship in less than a century. The motivation to examine, in a more detailed fashion, the motion of the Moon, may well have stemmed from this inexactitude.

Calibration of the perigee–apogee markers

Numerical coincidence may, once again, be cited, but it is a fact that $56=4\times 14$. The convenience of the Aubrey hole number allows the existence of a perigee/apogee marker-pair which could be moved at the simple prescription rate of 4 A.H. per lunation relative to the Sun marker. In Hoyle's tracking scheme, the Sun marker moves at the observationally corrected rate of 1 A.H. per 6.52218 d or 4.527716 A.H. per lunation. The net perigee marker movement rate is thus 0.527716 A.H. per lunation in the same direction as the Sun and Moon markers. This is equal to 6.5269 A.H. yr^{-1}, differing from the computed ideal rate of 6.3309 A.H. yr^{-1} by 0.1960 A.H. yr^{-1}.

If the Apogee marker were set into operation at the winter solstice of 3057 BC or 2871 BC, at this empirically determined rate, then 93 years later the perigee marker would be 18 Aubrey holes ahead of its proper position, after 10½ revolutions around the Aubrey circle. To assess the correct rate should have been a simple task to the geometers who designed the Stonehenge I structure.

Location and planning

Why Stonehenge? Or rather, why Salisbury Plain? As a site for a tide predictor it could hardly have been selected much further from the coastlines of what is now Southern England. But Stonehenge is not misplaced if consideration is given to the observational requirements. These may be listed as:

(1) Elevated plateau for an unobstructed view of the horizon.
(2) Level and treeless horizon, at least over the appropriate range of azimuths, so that the simplest relationship exists in the azimuth sequence.
(3) Horizon slightly elevated and not too distant, to avoid the worst effects of atmospheric refraction and absorption.
(4) Nearby land sloping slightly downwards away from the observing site to drain away winter groundmist, again at least over the appropriate azimuths.
(5) Not in the vicinity of a seacoast or of a large body of water, to avoid seamist or fog in winter.

That the Stonehenge site meets all these conditions may be an indication of the geographical coverage available to the designers. The Station Stones also indicate this, if their near-rectangular geometrical relationship really is due to a choice of latitude. The cultural aspects of these points have not been adequately discussed: the implication is of a cultural homogeneity of hitherto unsuspected extent.

The planning of the measurements and the choice of site certainly seem to have been justified by the practical execution of the observations. It now seems very likely that the causeway postholes represent, as Newham suggested, a sequence of measurements now that can be seen to culminate in the apogee moonrise azimuths of the winter solstices of 3057, 2871 and 2685 BC. The chance of accidental coincidence of azimuth, of the triple extreme astronomical event, and of the terminal postholes is slender indeed.

The ability to track such an elusive entity as the lunar apogee can be logically derived from the circumstantial evidence of Stonehenge I, as has been argued earlier. The later argument, of tidal prediction, is wholly speculative, being on a par with the eclipse prediction hypotheses, but contains the redeeming feature of practical motivation, unconnected with 'religion'. Moreover, the practical application is of a lower order of intellectual achievement than is the prodigious feat of designing and actually using a Neolithic lunar observatory. It is the latter for which we have evidence, and speculation on its purpose seems to me valid if there is required no great extension of the talents so clearly evident, five millennia after their application.

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Mantle origin of Cordilleran granites

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'Granite' batholiths of recent destructive margins have strontium isotopes inconsistent with crustal fusion. Associated enormous crustal thicknesses are produced without compressive shortening. These features necessitate accretion by vertical addition of predominantly mantle-derived calcalkaline intrusive and extrusive magmas.

ONE of the more controversial debates in contemporary earth science concerns the origin of granitic rocks, particularly those batholithic volumes characteristic of modern ocean–continent convergence zones. For some time the consensus of opinion was swayed by the weight of experimental evidence[1–3], accumulated over two decades, which shows that batholiths are derivable by partial melting at crustal pressures provided an ample source of metasediments is available. The overlap of granite melting conditions with granulite facies metamorphism of lower crustal regions is consistent with the extraction of such partial melts from their residua[4] leading to the two-layer crust typical of continents.

Theories of granite melting in the crust have been modified to explain the observed time–composition relations in batholiths which characteristically vary from early intermediate through to acid members of the granite family at later stages[5–7]. Detailed mapping in the Mesozoic and Cenozoic batholiths of Peru[8] has shown that minor cycles of this type, due to high level fractionation[9], are superimposed on a major cycle of primary differentiation starting with gabbro and terminating some 80 Myr later with adamellite. Considering the time span occupied by this primary cycle (note also California[6,10] and Alaska[11]) it is preferable to invoke a mechanism whereby separate batches of magma are generated by partial melting at successively higher crustal levels[12]. This conclusion follows from the crustal pressure experimental data: more acidic (and hydrous) melts are produced at lower pressures and temperatures. In the context of crustal melting, the most plausible way of having the highest melting temperatures

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recorded by the earliest intrusives is to suppose that crust–mantle interaction occurs at deep levels. Thus the origin of Cordilleran granites became linked to mantle processes (see also refs 7, 13–15) and I suggested[12] that rising andesitic magmas, generated within or above a subduction zone and either underplating or penetrating the crust, supplied essential heat and volatiles necessary to initiate granite melting at the deepest crustal levels. Once initiated, the granite cycle would be perpetuated with progressively more acidic contributions from shallower levels as the crust warms through from the base. Through this rather intimate association of potentially extrusive and intrusive magmas the time and space patterns of the latter are reconcilable, with a crustal anatectic origin. It follows that this evidence, and that involving time–composition relations, does not necessarily imply a mantle source for Cordilleran granite magmas which is where the strength of earlier arguments of this nature lay (ref. 14, for example). However, as field data and geochemical–isotopic observations have become more numerous, there have emerged two other persuasive lines of reasoning, considered below, which apparently place granite magma genesis at mantle depths.

The fractionation of large volumes of basic magma to produce granite batholith magmas[13] is not a popular theory because of the proportionately larger volumes of intermediate fractionates which are not found. Instead, these days the question we ask is whether relatively small percentages of basic to ultrabasic mantle melting or rather greater degrees of intermediate composition lower crust melting is responsible for granite magmatism.

Evidence from strontium isotope ratios

First we have the low initial strontium isotope ratios (^{87}Sr/^{86}Sr) which are emerging as a ubiquitous feature of Cordilleran granites. The values measured on a wide range of Western American granites range from 0.703 to 0.708 with the majority around 0.706 (refs 16–20), and values have been observed to increase with decreasing age in some Peruvian intrusives[21]. These ratios are a little higher than values being produced direct from the mantle in regions devoid of continental crust—a maximum present-day ratio of 0.704 in the case of Pacific oceanic basalts[22]. However, they are very much lower than contemporary values in crustal anatectic source regions containing ancient sialic basement or its erosional products. Values between 0.71 and 0.78, appropriate for such regions[8,16,23] depending on age and Rb/Sr ratio, seem to preclude an origin by direct crustal melting as described above.

Before accepting this as a rigorous conclusion, experience with strontium isotopes suggests we should beware of several ways in which the crustal melting hypothesis might be rescued. For example, guided by oxygen isotope work in the British Tertiary igneous province[24], it has become fashionable to reassess isotope patterns in terms of hydrothermal strontium transfer between intrusive and intruded rocks. It might be suggested that measured granite samples, usually taken from the exposed upper regions of batholiths, have acquired artificially low ratios due to equilibration with their mantle-derived andesite cover. However, in view of so many similar values having been determined from many different regions and erosion levels, I doubt that such an equilibration process is particularly important except in a local sense. The validity of strontium isotope interpretations can be checked by looking for ^{18}O depletions inherited from hydrothermal alteration[25], and the case for making parallel isotope measurements of strontium and oxygen in future work of this type is becoming recognised.

Perhaps a more significant method of producing low initial ^{87}Sr/^{86}Sr ratios by crustal fusion would be if the deep source region had a low Rb/Sr ratio. There is little disagreement about strontium concentrations in the continental crust and an average value of about 375 p.p.m. seems appropriate[26]. Crustal rubidium concentrations are more widely debated[26,27], but values close to the average adesite[28] (about 40 p.p.m.) seem appropriate and yield an average Rb/Sr ratio near 0.1. Bearing in mind the strong upward crustal concentration of rubidium[29], the incrementation rate of ^{87}Sr/^{86}Sr in buried crystalline basement might be less than 0.004 in 10^9 yr (corresponding to Rb/Sr=0.1). It follows that ratios normally ascribed to mantle could be derived from a relatively old lower crustal source region of low Rb/Sr. Of course, this incrementation rate is rather low in comparison to the mature ^{87}Sr/^{86}Sr ratios quoted earlier for exposed basement rocks, supposedly equivalent to those buried beneath younger orogenic belts, whose anatectic products would be distinguished easily.

At present this dilemma is not fully resolved but the temptation is to suggest that, since the Rb-poor basement rocks are usually in refractory granulite facies, the production of large volumes of low ^{87}Sr/^{86}Sr ratio granites from them seems most unlikely. This begs the question of how such rocks in the basement became deficient in rubidium and here it seems difficult to escape extraction by a melt phase leaving strontium partitioned in high temperature mineral residua[30] though this melt need not be indiginous and could be derived largely from the mantle. Shallow zones of unaffected old crystalline rocks, such as exposed in coastal Peru[8], would continue to accumulate radiogenic strontium by retaining their rubidium.

A final possibility is that underplating of the lower continental crust by low temperature, hydrous low ^{87}Sr/^{86}Sr mantle magmas, rapidly encountering their solidus temperatures, yields an immature zone later available for further partial melting (see refs 12, 14, 31). This merely splits granite magma generation into a two-stage process virtually indistinguishable from a direct mantle source on the grounds of strontium isotopes.

On balance, the conclusion to be drawn from strontium isotope observations is that Cordilleran granites contain a prominent component of recent mantle derivation. The style of granitic activity in relation to tectonic processes has doubtless changed considerably over the Earth's history[32,33], but voluminous granitic rocks generally record the isotopic imprint of recent mantle derivation from the early Precambrian[34], though fossilised early Phanerozoic Cordillera (for example Caledonian younger[35,36] but not necessarily older[37] granites), right up to recent times[16–21]. Lead isotopes provide further corroboration in that such data as are available indicate derivation of young granite and older granite gneiss leads direct from primary mantle growth curves[38,39].

Crustal thickness, deformation and accretion rates

Now let us examine a second line of attack on crustal melting hypotheses. From seismic and gravity data (refs 40 and 41, for example) it is well established that continental crustal thicknesses at destructive margins are much greater than average. In the central Andes, for instance, the crust reaches a maximum of 70 km thickness beneath the volcanic Cordillera[41], whereas 35 km is more typical of inactive regions. How does the crust locally acquire this great thickness? There are two possibilities: (1) crustal shortening whereby material is piled up due to laterally compressive forces produced where two plates are colliding and (2) thickening due to the addition of material vertically from the underlying mantle. Unlike continent–continent convergence zones, such as the Alps and Himalayas where strong compressive folding and overthrusting are in evidence[43], the field data from active ocean–continent boundaries, the sites of Cordilleran batholiths, reveal a striking lack of compressive tectonics. The intrusion of the Peruvian granites apparently created a tensional stress regime opening fracture systems which were the sites of block faulting, vertical movements[44] and even possibly crustal extension rather than shortening. The conclusion is that where two masses of like density converge the result is diastrophic whereas, at destructive margins, ocean crust and upper mantle are not forced down but dive quietly beneath continental crust with apparent ease.

Thus we have another significant difficulty for the crustal melting origin of granites. These rocks occupy about 25% of the crustal volume along destructive continental margins (average estimate based on various interpretations: refs 8, 41, 44–46) and, if we exclude thickening by crustal shortening, a large proportion of this material must be new crust recently derived by mantle melting. The other more positive candidates for derivation from the mantle are the extrusives, mainly andesites, but their volumes are too small to account for crustal thickening alone as they are essentially a veneer reaching only several km in thickness at maximum[47]. These concepts are best illustrated by a simple numerical estimate of the modern rate of crustal evolution involving both contemporary intrusives and extrusives. As a type example we can use the young Peru Coastal Batholith region, for which we now have abundant data[5,8,9,19,20,21,40,41,44,47]. The volume of crust produced by igneous events in this region during the last 10^8 years can be estimated at 1.2×10^6 km^3, based on a width of 60 km normal to the margin, a length of 1,000 km in Peru, and a material thickness of 20 km. The rate of crustal evolution from igneous activity in Peru is therefore about 0.012 km^3 yr^{-1} where "evolution" includes any recycled products of crustal fusion in addition to new material.

Now, in terms of crustal growth Peru was chosen because it probably represents an average accretion rate between the more dramatic effects of intracontinental mountain building, despite crustal shortening, and the lesser volumes involved in island arcs. To advance the argument we can estimate the contemporary crustal evolution rate at $0.012 \times 40{,}000/1{,}000 = 0.5$ km^3 yr^{-1} as I am persuaded (R. Oxburgh, personal communication) that we should consider about 40,000 km length for the world's recently active subduction zones and recently developed intracontinental margins. Again, this rate would include any recycled crustal fusion products. Obviously there will have been some erosion over the time spanned, allowance for which would slightly increase the rate estimated above. However, the amount will be too small to be significant in this approximate calculation, especially as much of the eroded material is reconstituted as continental crust by uplift at intracontinental collisions or by accumulation on the continental shelf. (For example, the Atlantic bordering shelf regions receive a surprisingly large proportion of the Andean erosion products because of eastward drainage[48]—relatively little sediment accumulates in the Peru–Chile trench[40].)

Accepting that crustal evolution is occurring at about 0.5 km^3 yr^{-1}, it follows that if all this is new material added to the crust, in which case our evolution rate becomes the crustal growth rate, 10,000 Myr would be required to produce all 5×10^9 km^3 of continental crust which now exist. Of course, it seems highly likely that accretion rates would have been rather higher in the past—a time-average of 1.1 km^3 yr^{-1} is required over the Earth's history[12]—and our modern figure of about half the time average is consistent with the decay of long-lived radioisotopes having reduced modern heat productivity to about a quarter of its initial value[49].

If we drop crustal growth rates to include only the extrusive component of destructive margins then a rate nearer 0.1 km^3 yr^{-1} would result, because intrusives predominate over extrusives. The observed crustal thickening at destructive margins could then not be achieved without severe shortening, and it is also difficult to see how continents could even have reached their present volumes without disproportionately rapid early accretion rates. Unfortunately, numerical estimates of accretion rates can never be better than highly approximate. Yet on balance, the evidence from thickening and crustal growth again demands that Cordilleran granite magmas be derived from mantle depths, whether this is by a direct melting and emplacement event or by the indirect two-stage process mentioned earlier.

Andesites, granites and mantle melting

If granite batholiths owe their origin to mantle melting, we should return to the experimental data to consider whether the observed time–space–composition relations can be produced in this revised context. In order to produce magmas over the entire lifespan of a destructive margin it is necessary to continuously replenish the source region or igneous activity would have limited life. Some involvement of subduction zone materials is implied either as a source of melt or of volatiles capable of melting overlying mantle[50].

A good starting point is to consider andesite genesis and to extend the argument later for granites. High pressure experimental studies[51], ranging up to 40 kbar, indicate that primary andesite magmas can be produced by partial fusion of subducted, amphibolitised ocean crust[31,52] corresponding to quartz eclogite[15] after dehydration. However, certain other difficulties have been encountered in generating andesites by simply melting subducted ocean crust[53,54] since 25–50% partial melting would be required to account for both the SiO_2 content and Fe/Mg ratios of average andesites. This degree of melting would dilute incompatible, large ion lithophile (LIL) trace elements yielding concentrations several times lower than observed even assuming their complete partitioning into the melt phase. Consequently, it has been necessary to devise rather more sophisticated mechanisms to account for andesite geochemistry and basically there are two possibilities: (1) Magma generation occurs within the subduction zone, satisfying major element andesite requirements, followed by enrichment in LIL elements during ascent by scavenging[56] of the overlying refractory ultrabasic mantle and, possibly, lower crust (2) The hydrous subducted lithosphere dehydrates[31,57] releasing volatiles, already enriched in LIL elements, into the overlying dry ultrabasic mantle causing incipient melting there. Only 5–10% melting is required in this case to produce andesites which are more likely to satisfy both major and minor element constraints[54].

Following Ringwood[15], Thorpe *et al.*[55] have provided an interesting bridge between these two ideas by deriving a garnet pyroxenite LIL-rich wedge over the subduction zone which is later able to melt giving andesite. This is a continuous process active so long as subduction continues. In this way, the variable initial $^{87}Sr/^{86}Sr$ ratios for andesites are produced[47] as radiogenic strontium accumulates for different times in a temporarily Rb-rich source region. However, Spooner[52] has shown that some ocean crustal rocks may increase their $^{87}Sr/^{86}Sr$ ratios by hydrothermal exchange with seawater already enriched in a continent-derived strontium component. Involvement of such materials in the production of melt beneath a Cordilleran margin provides a source for initial strontium isotope variability directly, without necessitating a complex mechanism.

It seems that a mechanism for producing andesite magmas at mantle depths is evolving, but what of granite magmas? At first sight there are problems because partial fusion of upper mantle materials cannot directly produce acid liquids[13,51]. However, if one defines the term "granite" more rigorously it is immediately obvious that the average composition of most batholiths lies between quartz diorite (tonalite) and granodiorite, rather than granite in the strict sense. Indeed, if we ignore the limitations of rock nomenclature based on grain size, the similarity between the material in batholiths and volcanic andesites on geochemical grounds is quite striking. W. S. Pitcher (personal communication) and Myers[58] have commented on these geochemical similarities in Peru: increasingly it is becoming evident that many of the intrusive centres are fossilised magma conduits which fed the overlying extrusives. Apparently tonalite (that is, andesite) magmas rose by stoping and cauldron subsidence towards the surface, locally generating a varied compositional suite by the late stage secondary differentiation process described earlier. Some assimilation or partial melting of crustal material en route to the surface is probable, and is supported by the secular changes towards higher initial strontium isotope ratios during mid-Cretaceous to Quaternary Andean plutonic activity[21]. Indeed, this process may be an essential part of producing a refractory, Rb-poor basement. Crustal melting experiments are relevant here, and show that mantle-derived tonalite melts should enter the crust with enough thermal energy gradually to drain the lower crust of near minimum melting components. I would attribute the primary composition trend (see introduction) on a time scale of 10^8 years in Cordilleran batholiths to the gradual raising of crustal temperatures during the active cycle so that both mantle magmas can assimilate at higher crustal levels to become relatively granitic, and these more granitic magmas can reach higher levels before being trapped by their melting curves[12,59]. Of course, crustal assimilation is limited by the constraints discussed earlier in this paper, but an andesitic magma need only increase its volume by between 25 and 50% to resemble the most acidic, volumetrically significant adamellites of Peru. As more data become available further small changes towards systematically higher initial ^{87}Sr/^{86}Sr ratios (see ref. 21) should be traced through the primary time–composition trend of Cordilleran granites.

The consanguinity of intrusive and extrusive magmas at destructive plate margins seems to remove the experimental objections to furnishing batholiths at the expense of mantle. The arguments given in this paper suggest that those who advocate exclusive derivation of Cordilleran granites by reprocessing of existing continental crust should seriously contemplate a revision of their thoughts. The onus is on them to show how Andean-type crustal thickening and low initial ^{87}Sr/^{86}Sr ratios in Cordilleran granites are produced without mantle involvement.

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Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies

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It is shown that electrophoretic genetic distance estimates are highly correlated with albumin immunological distances between the same pairs of species. The bimodality of rates of electrophoretic differentiation at various loci is then documented and the electrophoretic clocks involved are calibrated. The differences between the systematics and population genetics uses of electrophoretic data are then discussed. Finally, a direct electrophoretic test of the neutral alleles hypothesis is presented and exemplified on a set of Thomomys bottae-umbrinus *data.*

GENOMIC differentiation between diverging lineages begins as soon as their gene pools have developed a sufficient degree of reproductive isolation. Recently it has become possible to provide direct measures of the degree of such differentiation through the comparative biochemical techniques of amino acid sequencing, DNA hybridisation, electrophoresis, and immumology. As amino acid sequencing is much too time-consuming for routine application to evolutionary studies, and DNA hybridisation untested in detail, most of our current estimates of biochemical genetic distances derive from electrophoretic and immunological comparisons of proteins from species of interest. Electrophoresis, in particular, has become the favourite tool of those wishing to apply protein data to problems in systematics and population biology. Nonetheless, few, if any, studies have been carried out to develop an appropriate molecular context within which the meaning of the electrophoretic data could be properly comprehended. We especially lack direct information as to the rates of amino acid substitution in the various proteins used in electrophoretic studies, which makes it difficult to tell whether the calculation of a single genetic distance from data involving numerous loci is a legitimate exercise, and, if so, how to relate these distances to a possible time scale. Our approach to this problem began with a comparison of electrophoretic and immunological distances for the same pairs of species. The results of that study led to an appreciation of the striking bimodality in the observed rates of protein electrophoretic evolution, of the ready availability and use of the set of rapidly evolving proteins to easily deal with levels of relationship heretofore inaccessible to the molecular systematist, and, finally, to the development of a direct test of the neutral alleles hypothesis in explaining electrophoretic polymorphisms.

We can begin with a definition of the distance measures used. For electrophoretic comparisons, Nei has introduced a measure of genetic distance, D, where D is the average number of electrophoretically detectable substitutions per locus between the two populations being compared[1,2]. If x and y are the frequencies of the i allele in populations x and y, then the measure of genetic identity (I) is given by the equation

$$I_{xy} = \frac{\Sigma x_i y_i}{(\Sigma x_i{}^2 \Sigma y_i y_i{}^2)^{1/2}}$$

where the summation is over all alleles and all loci compared, and $D = -\log S$.

For the immunological comparisons, we use the microcomplement fixation immunological distance (ID)[3], where the ID between two homologous proteins is equal to five times the % sequence difference between them[4]. For the proteins whose immunology will be discussed here, albumin and transferrin, one ID unit will then be equivalent to about one amino acid substitution.

Electrophoretic genetic distance and time

The albumin immunological, and Nei electrophoretic, distances for 76 pairs of species were measured and compared (Fig. 1). There is obviously a strong correlation ($r = 0.82$) between the two measures of genetic distance, which, since different loci are being studied, suggests that each is a function of a third variable —most probably time. Ample documentation of the time-dependent nature of the vast majority of amino acid substitutions in a variety of organisms has been made in the last 15 years[10,11]—so that, for example, one can speak of an albumin 'clock', and document that it generally keeps rather good time[12]. Any such clock is necessarily a statistical one, and the comparison of two of them—in this case, the albumin and electrophoretic clocks—has to be carried with this fact well in mind. Each point in Fig. 1, considered in a time dimension, then becomes a probability ellipse. Because the number of substitutions counted in albumin immunological comparisons is appreciably greater than the number counted for the corresponding typical electrophoretic study involving 20–25 loci, the long axis of the ellipse will be parallel to the D axis. This is approximated in Fig. 1, where the dashed lines encompass an area within one electrophoretic standard error[1] of the regression line. The standard error is given by

$$\text{s.e.} = \left(\frac{1-I}{nI}\right)^{1/2}$$

where n is number of loci. To a first approximation, then, if the albumin and electrophoretic clocks were keeping perfect time, 2/3 of all the points should fall within the dashed lines. Remarkably enough, except for the cluster of seven points involving the kangaroo rat species *Dipodomys ordii*, they do. For *D. ordii*, presumably, relative to the actual phyletic distance separating it from other kangaroo rats, the albumin distance is uncharacteristically low or the electrophoretic distance too high. Immunological studies of *Dipodomys* and other geomyoid transferrins[7] give a distance between *ordii* and other *Dipodomys* species in line with the electrophoretic distance, suggesting that in this case it is the albumin immunological distance which is misleading.

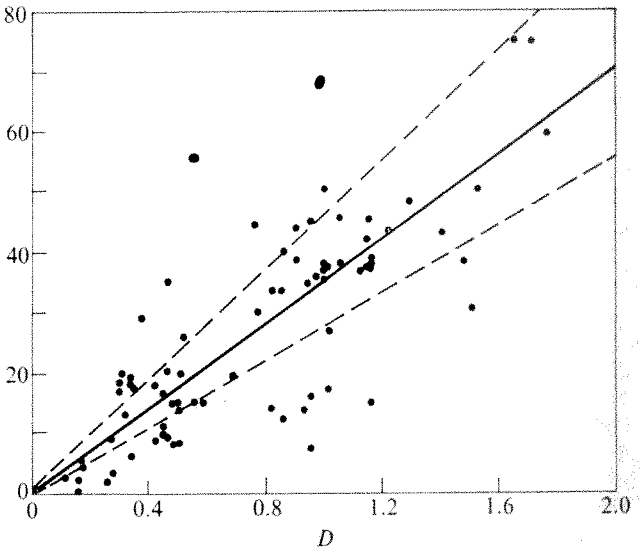

Fig. 1 Microcomplement fixation immunological distances are plotted against the electrophoretic Nei D values for the same species pairs. A preliminary version and discussion of these data have been presented previously (V.M.S., L. R. Maxson and A. C. Wilson, paper presented to the Society for the Study of Evolution, December 1973). The comparisons use published electrophoretic data on *Dipodomys*[5], *Peromyscus*[6,7], *Hyla*[8], and *Anolis*[9]. Other electrophoretic data on *Sigmodon* were provided by W. E. Johnson and R. K. Selander, on *Anolis* by G. C. Gorman, and on *Thomomys* by S. Y. Yang and J. L. Patton. All of the albumin immunological comparisons were carried out in this laboratory. The dashed lines indicate the standard errors of the electrophoretic D values. $r = 0.82$ (76 points).

It is thus evident that there is an electrophoretic clock and that it can be calibrated in terms of the albumin clock—not so much, it must be emphasised, because of earlier demonstrations of the strongly time-dependent nature of albumin evolution in other taxa, but because the rate of albumin evolution is known from those studies. Albumins accumulate some 100 units of immunological distance for every 60 Myr of separation[12], and, from Fig. 1, AID $= 35D$. Thus it takes, on the average, 20 Myr for a typical locus to accept an electrophoretically detectable substitution in one or the other of a pair of diverging lineages. Assuming a mean size of 400 amino acids[13], and that 20% of all substitutions are electrophoretically detectable, this means that the average protein sampled in the usual electrophoretic studies has a unit evolutionary period[10] in the area of 15–20 Myr. This very large figure is characteristic of the classic slowly evolving proteins such as cytochrome c and glyceraldehyde 3-phosphate dehydrogenase, and this makes one wonder as to the representativeness of these loci—especially when it comes to calculating times of divergence from electrophoretic data. For example, Nei's original calibration[1], which has been used in numerous divergence time calculations (see Table 2), set a D of 1 at about 3 Myr of separation—a figure almost an order of magnitude too low. An important complicating factor, however, has to be dealt with before realistic time of divergence calculations can be made.

Locus-dependent rates of change

This problem was first evidenced in the findings of King and Wilson for the *Homo-Pan* electrophoretic comparison[14,15]. There the D value of 0.29 for the various 'intracellular' loci sampled stood in sharp contrast to the value of 2 for the plasma protein

Table 1 Plasma protein D values and albumin plus transferrin immunological distances between the same pairs of taxa

Taxa compared	Albumin plus transferrin distance	Plasma protein D
Cercopithecus patas–C. aethiops	3	0.4
Papio–Theropithecus	7	0.6
Pan paniscus–P. troglodytes	7	0.7
Macaca nigra–M. fascicularis	9	0.7
C. patas–C. talapoin	14	1.5
Pan–Gorilla–Homo	15	1.6
M. nigra–Papio	22	2.0
Cercocebus albigena–C. torquatus	26	2.0

loci. That this result is not a statistical fluke is documented in Table 1. We list there several plasma protein D values and the corresponding albumin plus transferrin immunological distances. As transferrin accumulates amino acid substitutions some 50–60% more rapidly than albumin[16,17], one thereby obtains immunological distances sufficiently large to be reliably compared to the plasma protein D values. For the data of Table 1, the summed immunological distance corresponding to a D value of 1 is about 10 units, of which about 4, on the average, will have been contributed by the albumins. Thus the *Homo-Pan* result, if anything, slightly underestimated reality, and the plasma proteins are accumulating amino acid substitutions some 10 time more rapidly than the enzymes sampled in the electrophoretic surveys which provide the bulk of the data of Fig. 1. Nor do we see evidence to date of any particular conservation in the rate of electrophoretic change in any particular plasma protein (for example, the plasmas of *Homo*, *Pan*, and *Gorilla* show only one band with the same mobility in all three); thus we are forced to the conclusion that a rather striking bimodality describes the distribution of electrophoretically measured rates of evolution at the various protein loci. In other words, there are two sets of proteins, and one of them is accumulating electrophoretically detectable substitutions at a rate tenfold greater than the other. This rapidly evolving set includes, in addition to the plasma proteins, various other 'secreted' proteins such as those found in saliva, egg-whites, and haemolymph, the nonspecific esterases, and certain enzymes not involved in complex metabolic pathways—such as ribonuclease, lysozyme, and carbonic anhydrase; that is, many of the class 2 and 3 loci[18-20].

Setting the electrophoretic clock

This finding, as we shall see, has certain very important corollaries, not the least of which is the proper setting of the electrophoretic clock. As noted above, numerous authors have recently attempted to date divergence events among populations using electrophoretic data. These data typically, however, involve comparisons among both slowly and rapidly evolving proteins—with the latter usually including albumin, transferrin, haemoglobin, and one or more esterases. Clearly the findings reported here require a partitioning of the electrophoretic data set into two, as the clocks involved are obviously moving at grossly different rates. In essence, then, early in the process of genetic differentiation between two diverging lineages the bulk of any measured distance will be contributed by the rapidly evolving loci. This contribution will be essentially complete by 5–6 Myr of separation (that is, all the rapidly evolving proteins will have different electrophoretic mobilities by that time), and any further accumulation of genetic distance will be seen only in the slowly evolving loci. Future studies then ought to focus on a large set of rapidly evolving loci for temporally close relationships, and on a comparably sized set of slowly evolving loci once one gets beyond 4–5 M yr of separation. Specific divergence times can then be calculated once the equation AID $= 35D$ is corrected for the fact that the data from which it was derived stem from studies averaging about 3/4 slowly evolving loci and 1/4 rapidly evolving. At a D of 1, $I = 0.37$, and effectively all this similarity will have been contributed by the slowly evolving loci—thus their actual I value will be 0.37/0.75, or 0.5; corresponding to a D of 0.7. That is, in 35 M yr the set of slowly evolving loci will give a D value of 0.7, so that the corrected equations will approximate: (1) slowly evolving loci: AID $= 50D$; $T(\mathrm{Myr}) = 30D$ (2) rapidly evolving loci: AID $= 4D$; $T(\mathrm{Myr}) = 2.4D$.

We include, for heuristic purposes, a plot relating time of separation to D for a typical study where 3/4 of the loci are slowly evolving, and 1/4 rapidly evolving, as Fig. 2. In addition, we present in Table 2 some adjusted times of divergence involving data already in the literature and using the calibrations derived in this article. It might be noted that some of those adjustments produce substantial change which will have to be taken into account when reassessments of the evolutionary pictures implied are made.

Systematics against population genetics—sample sizes

At this point, before getting into more theoretical matters, it is appropriate to consider the distinction between population genetics and systematics uses of electrophoretic data. The basic interests of the population geneticist of course lie in the realm of population dynamics, and the knowledge of allele frequencies is an essential prerequisite to further work. The population geneticist, then, studies populations. The systematist, on the other hand, has as a first goal the knowledge of the evolutionary relationships among the organisms with which he is working. Thus, in assessing, for example, the evolutionary genetic distance between humans and chimpanzees, one human and one chimpanzee can give an entirely representative picture. The question therefore arises as to how many individuals per population will be necessary for a particular study, and clearly the answer to this question depends on the genetic distances involved, as well as on the rates of evolution of the loci being sampled. It then becomes vital to achieve some reliable assessment of the nature of the evolutionary relationships involved in the study at hand before, for example, undertaking the onerous and time-consuming task of surveying numerous populations with many individuals per population at 20 or more loci—as has all too typically been the case in much recent electrophoretic work. Such an assessment has to take place in stages, and it might be instructive to illustrate those stages with specific examples.

The first stage question would simply ask whether effective genetic isolation has been achieved between the populations in question. As the genetic differentiation which accompanies the development of such isolation will most readily be seen here with the set of rapidly evolving loci, one should first compare the various easily available secreted proteins—for vertebrates, this would mean the plasma proteins and the nonspecific esterases.

Table 2 Some published times of divergence calculated from electrophoretic data and adjusted according to the calibrations introduced in this article

Taxa involved and specific divergence event	Proportion fast loci	I	Published divergence time (M yr)	Calculated divergence time (M yr)	
Icteridae (blackbirds)					
Agelaiine–Quiscaline groups	0.53	0.7	0.27	1.4	ref. 21
Thomomys talpoides (gophers)					
48-chromosome New Mexico populations and others	0.36	0.845	0.23	1.2	ref. 22
Peromyscus (deer mice)					
boylei and *truei* species groups (excepting *polius*)	0.47	0.68	0.24	2.1	ref. 23
Drosophila					
virilis–littoralis–montana species groups	ca0.7	0.37	0.75	3.9	ref. 1

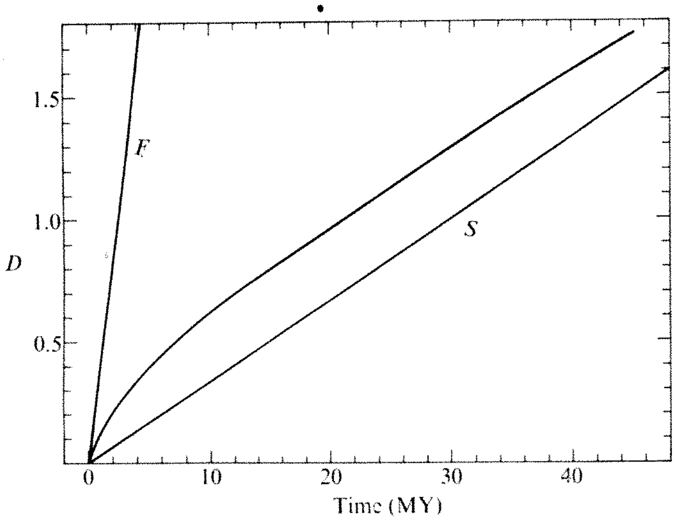

Fig. 2 The accumulation of electrophoretic genetic distance (*D*) as a function of time for the rapidly evolving proteins (F), slowly evolving proteins (S), and a mixed system involving 3/4 slowly evolving, and 1/4 rapidly evolving proteins (unlabelled line) calculated according to the calibrations discussed in the text.

With reasonably fresh material one ought to be able to readily see some 25–30 bands on a single gel. These could be compared most simply in two individuals as the number with the same mobility divided by the number counted in both. Thus the only way that two individuals drawn from the same gene pool could give a *I* value of less than 1 would be for them to be homozygous for different alleles at one or more loci. The maximum probability for such an occurrence is 1/8 (at a polymorphic locus with two alleles at a frequency of 0.5)—thus even if 1/3 of our 25–30 loci were polymorphic, it would be unlikely for the two individuals to be counted as different at more than one locus. Even this rather low 'noise' level can be effectively eliminated by comparing two individuals per population—the 1/8 probability per locus then drops to 1/128. Beyond this one gains nothing until a large enough number of alleles is counted so that one can reliably speak of allele frequencies, and the required 30 or so individuals now increases the workload by more than an order of magnitude without anything approaching a commensurate gain in effective resolving power. In fact, if one takes into account observed heterozygosity values and the evolutionary rates calculated here, it becomes evident that very little indeed in the way of resolving power is contributed to the systematics of a group by that component of genetic distance due to allele frequency differences at the slowly evolving loci. Resolving power over the last few million years of relationship is far more efficiently gained through the use of the rapidly evolving set of loci—most simply by the band counting approach just outlined, and ultimately by allele frequency comparisons at those loci which are polymorphic. Appropriate attention to these considerations, with emphasis on increasing the number of slowly evolving loci in the study, should then greatly facilitate routinely working with evolutionary relationships extending over the last 50 MY or so ($D_{\rm slow} = 1.6$). If, for example, one could routinely work with 40 slowly evolving loci, this would mean that the effective rate of electrophoretic substitution along any given lineage is one per 1.5 Myr; that is, the average ultimate resolving power is 1.5 Myr up to about 50 Myr of separation. It is, of course, 10–50-fold better in the range 0–6 Myr due to the ready availability of a large set of rapidly evolving loci.

A practical test of this 'band-counting' approach to the electrophoretic systematics of closely related forms is available among the kangaroo rats (*Dipodomys*). Patton, MacArthur, and Yang have recently reported[24] that *Dipodomys heermanni* is clearly composed of two quite distinct species, with one more closely related to a third, *D. panamintinus*, than it is to the other *heermanni* (now renamed *californicus*). That study surveyed 22 loci and some 100 individual kangaroo rats. Yet one gel with one individual per population at issue—using band-counting for the plasma proteins and liver esterases—can give congruent results; although, of course, the *D* values obtained are appreciably higher (Table 3). It should also be noted that for many taxa of interest populations are not realistically available, and this approach enables one to learn a great deal about relationships at taxonomic levels heretofore mysterious using sample sizes of only one or two individuals per population. Such a preliminary effort among the Old World monkeys, complementing an already completed immunological study, has already been reported[25]. We have also been able to obtain the first reliable measures of genetic distance separating the pygmy chimpanzee (*Pan paniscus*) from the more common *Pan troglodytes*, and the Eastern and Western lowland gorillas. In each case the plasma protein *D* value was 0.7, suggesting separations on the order of 2 Myr ago. The corresponding *Pan-Homo-Gorilla* distances are 1.6–1.7, consistent with the summed albumin and transferrin immunological distances among the three of 15 units (Table 1).

These results also suggest that some degree of caution should be exercised in the use of genetic distance criteria to assess the taxonomic levels at which populations ought to be separated. Obviously the genetic distances obtained will be very dependent on the loci included in the study, and a 'species level' *I* value of 0.85 for a study involving a preponderance of slowly evolving loci might well become a 'genus level' value of 0.3 when looking at the rapidly evolving set. Thus the *D. heermanni–D. panamintinus* *I* value of 0.9 (ref. 24), which might be used to argue for conspecificity, becomes 0.6 when the plasma proteins and liver esterases are used. One can, however, foresee a possible negative effect of this newly increased resolving power when dealing with lineages of relatively recent origin. This will stem from the marked proliferation of recognisably different (at the genetic level, at least) populations which are not exchanging genes (that is, which have fixed different alleles), and which, therefore, can be seen as representing different 'species'. For mammals, this proliferation will certainly occur among the rodents—and one can only hope that this increased resolving power might be used to raise our level of understanding of the dynamics of the evolutionary process, and not to make it so much easier to play the 'splitting' game in formal taxonomy.

A direct test of the neutral alleles hypothesis

Finally, we can consider the test of the neutral allele hypothesis. We know that, for a neutral allele polymorphism, the length of time a given lineage remains polymorphic will depend only on the size of the populations along it. The probability of a given locus being polymorphic, on the other hand, is a function of the rate of amino acid substitution at that locus. Thus rapidly substituting loci will be polymorphic more often, thereby being more likely to contribute to population heterozygosity—again, to emphasise, for neutral allele situations. For such situations, then, heterozygosity per locus will be proportional to the rate of amino acid substitution at that locus. To the extent that this is actually descriptive of a body of data, we argue that such a data set is consistent with the neutral hypothesis; to the extent that it is not, we are encouraged to look for specific selective effects. One case where the neutral hypothesis does seem entirely adequate to explain the observed data involves a study of 25 *Thomomys bottae-umbrinus* (pocket gopher) populations, where at least 30 individuals per population were surveyed at 23 loci

Table 3 A comparison of the results obtained with the plasma proteins and liver esterases using one individual per population with those obtained in the usual population genetics approach in *Dipodomys*

	D.h.(5)	D.p.	D.h.(4)
Dipodomys heermanni (5-toed)	0	0.47	1.60
Dipodomys panamintinus	0.08	0	1.35
Dipodomys heermanni (4-toed)	0.24	0.15	0

The numbers above the diagonal are the plasma protein plus liver esterase *D* values using one individual per taxon. Those below the diagonal were obtained using 18 'slow' and 4 'fast' loci with a total of 100 individuals[24].

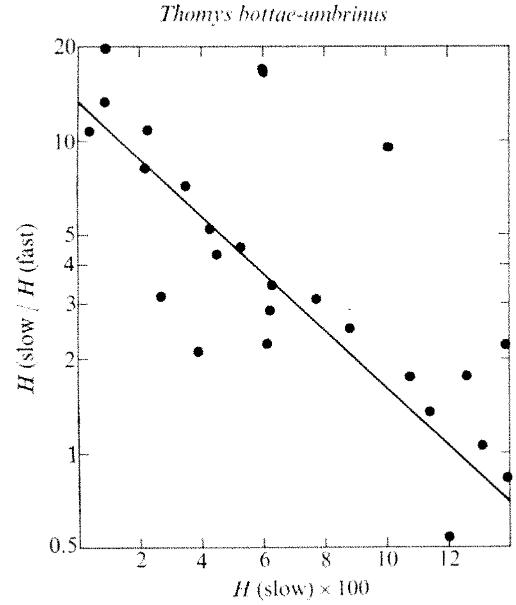

Fig. 3 The ratio of heterozygosity (H) values in a series of *Thomomys bottae* and *umbrinus* populations for sets of 18 slowly evolving, and 5 rapidly evolving loci are plotted against the H values for the slowly evolving set. The justification for the log-linear plot is presented in the text.

(18 'slow', 5 'fast')[25]. The H values for the 'fast' and 'slow' sets of loci were then calculated, and plotted as log H (fast)/H(slow) against H(slow) for each population (Fig. 3). The log transform is to allow for the fact that the number of potentially detectable electrophoretic alleles in a population is limited by the integral nature of charge changes—thus the H(fast)/H(slow) ratio could not increase as a linear function of H(slow). It should be noted that H(fast) clearly does increase as H(slow) increases and, extrapolating to $H(\text{slow}) = 0$ gives us a H(fast)/H(slow) intercept of 13, which is, considering the statistical uncertainties involved, remarkably similar to the D(fast)/D(slow) value of about 12 derived previously. It is difficult to see how this quantitative congruence between rates of substitution and heterozygosity levels at particular sets of loci could be the result of anything other than the random walking of neutral alleles.

Summary and suggestions for further work

These findings should be considered indicative of an area in which a great deal of further work needs to be done, and not necessarily representative of a general pattern. The data of Fig. 1 certainly argue forcibly for the strongly time-dependent nature of rates of electrophoretic differentiation, but there is, nonetheless, adequate space left for a certain degree of selective maintenance of various polymorphisms. Appropriate further testing of the neutral allele hypothesis in the manner just described awaits a sufficiently large sampling of rapidly evolving loci so that the sample sizes of the two sets of loci are comparable. This should be a relatively simple matter in most vertebrate groups, where one should certainly be able to routinely score 15 plasma protein, and 5–10 esterase, loci. For the very large and important *Drosophila* data set, however, with which so much of the neutralist-selectionist controversy has been concerned, it will be necessary to document a reasonable sample of rapidly evolving loci prior to carrying out these tests. Presumably these would be most readily available in the haemolymph and among the larval proteins and non-specific esterases, though clearly the rates of differentiation at the various enzymatic loci ought also to be compared to see if any of those are especially rapidly evolving. The available data, then, are explicable within a simple framework involving a very small number of variables, and the next step consists of expanding the taxonomic base within which this simple framework can be seen as operative.

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T4 Bacteriophage-coded RNA polymerase subunit blocks host transcription and unfolds the host chromosome

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T4 bacteriophage mutants selected for their ability to grow with cytosine in their DNA are defective in host transcriptional shutoff and host chromosome unfolding. The host RNA polymerase purified from cells infected by such a mutant lacks a small T4-coded subunit.

T4 BACTERIOPHAGE shuts off host transcription efficiently and rapidly but the molecular mechanism behind this remains a mystery. One reason for the mystery may be that no T4 mutants defective in host shutoff have yet been isolated. Since T4 probably uses the host RNA polymerase for all its transcription[1,2], inactivation of the polymerase cannot be involved in shutoff. During infection however, various T4 coded polypeptides bind to the host RNA polymerase. These include the products of T4 genes *55* (refs 3 and 4) and *33* (refs 3 and 5) and of two other unidentified genes. The latter two polypeptides, molecular weights 15,000 and 10,000, were assigned the numbers 2 and 4, respectively, by Stevens[3]. There are other polypeptides which bind loosely to the RNA polymerase[6]. In addition, the α subunit of the RNA polymerase is adenylated[7] by two distinct T4-coded activities, *alt* and *mod* (ref. 25), one of which, *alt*, is carried in the virus particle[8,9]. The adenylated RNA polymerase transcribes *E. coli* genes poorly *in vitro*[29]. Neither *alt* nor *mod* mutants affect the shutoff of host protein or r RNA synthesis *in vivo*[8], however, and none of the T4-coded RNA polymerase subunits has been implicated in host shutoff.

Besides changing the host RNA polymerase, T4 infection alters the host "chromosome". The host DNA changes its intracellular location[31] (nuclear disruption), a process requiring the T4 *ndd* gene product[10]. The highly compact structure of the host nucleoid is unfolded[12], requiring the product of the T4 *unf* gene[11]. The host DNA is degraded to mononucleotides chiefly by the products of genes *denA endoII* (refs 13 and 14) and *46*, *47* (ref. 15). All of these phenomena can proceed independently of each other[10,11]. None of them has been related in a simple way to the shutoff of the synthesis of at least the major host proteins[11].

T4 has hydroxymethylcytosine in its DNA instead of the usual cytosine[16]. It is tempting to speculate that the shutoff of host transcription and/or replication occurs when T4 causes the cell to reject DNA with cytosine for some aspect of transcription and/or replication. If this is true, T4 with cytosine in its DNA might "shut itself off". T4 mutations which permit the multiplication of cytosine-containing T4 would occur in genes normally involved in host shutoff. To test this hypothesis, and to find T4 regulatory genes, we have begun the search for T4 mutants which can produce T4 with cytosine in their DNA.

The first such mutants we have found are deficient in the function of a gene which we call *alc* (ref. 17). When cytosine-containing T4 DNA is made after infection, the *alc* gene product almost completely prevents true-late transcription. True-late mRNAs are those which require T4 DNA replication[18] to have occurred and the products of genes *33*, *55* (ref. 18) and *45* (ref. 19) to be present. Here we present evidence that the product of the *alc* gene becomes an RNA polymerase subunit, probably the polypeptide no. 2 of Stevens[3]. It is also required to unfold the host nucleoid after infection and is likely to be the *unf* gene product of Snustad and his collaborators[11]. We discuss the implications of this for the structure of the host nucleoid, and the relationship of the structure to transcriptional regulation.

Missing T4-coded polypeptide in *alc* RNA polymerase

In the original report on *alc*$^-$ mutants, we proposed that the *alc* gene product, itself non-essential, interacts with an essential part of the transcription apparatus. We proposed that this may be one of the activities which adenylate the α subunit of the RNA polymerase or may be one of the T4-coded RNA polymerase subunits. Since mutants are available which lack one or the other adenylating activity[8], we tested these first. Neither *alt*$^-$ nor *mod*$^-$ mutants were able to confer the *alc*$^-$ phenotype after recombination with T4 which makes cytosine-containing DNA. Thus, we tentatively concluded that *alc*$^-$ mutants are not simply *alt*$^-$ or *mod*$^-$. We then purified the RNA polymerase from cells after infection by an *alc*$^-$ mutant to test for the presence of the T4-coded polypeptides. Figures 1*a* and *b* show polyacrylamide gels of RNA polymerase from *alc*$^+$ and *alc1* (*alc*$^-$) infected cells respectively. The *alc*$^-$ mutant is conspicuously lacking the major peak of radioactivity in about fraction 33, but has all the others. By molecular weight determinations with known markers and by comparison with the published results of Stevens[3], we think that the missing peak is the 15,000 molecular weight polypeptide no. 2. *Alc1* was a spontaneous mutant of the *alc*$^+$ parent shown in Fig. 1*a*. Thus, we are quite sure that the *alc*$^-$ mutation caused the absence of the polypeptide in the RNA polymerase. In addition, we have done this experiment using 'mixed labels'. The *alc1* mutant infected cells were labelled with ^{35}S-methionine and the *alc*$^+$ parent with ^{3}H leucine. The infected cells were then mixed and the RNA polymerase purified as before. The polypeptide in question was only labelled with ^{3}H offering additional evidence that, after infection by the *alc1* mutant, the polypeptide is not bound to the RNA polymerase. This is not true for all apparent *alc*$^-$ mutants, however. We shall return to this point later. The *alc1* mutation does not seem to affect the synthesis of any other T4 early gene products. Thus, *alc* is probably the structural gene for this RNA polymerase-bound polypeptide.

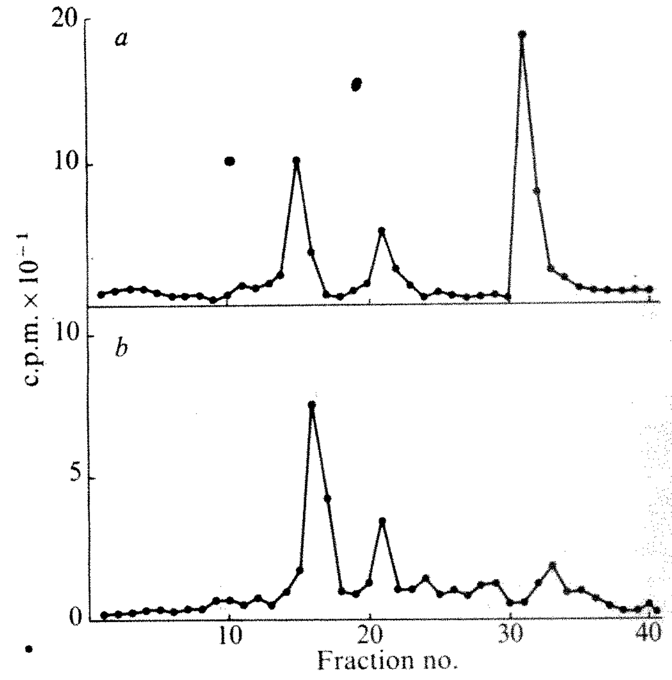

Fig. 1 An *alc*$^-$ mutant is lacking a T4-coded polypeptide on the RNA polymerase. *E. coli* B$^{\text{E}}$ (100 ml) growing in M9 without amino-acids at 30 °C were labelled with ^{3}H-leucine 5–15 min after infection. They were then mixed with 1 g of uninfected cells, sonicated, and the RNA polymerase purified by the method of Burgess[21] through phosphocellulose chromatography. The results of slicing and counting 12% SDS gels (procedure of Fairbanks *et al.*[22]) is shown. The dye front was on the right. *a*, T4-coded polypeptides on the RNA polymerase from the parent of the spontaneous *alc*$^-$ (alc1) mutant (*b*). The *alc*$^-$ mutant is clearly missing the first peak from the right in about fraction 34. We think this peak is due to Stevens' polypeptide no. 2.

Map position of *alc*$^-$ mutations

The *alc* gene product seems to be relatively non-essential in most laboratory conditions. We have found, however, that the multiplication of some *alc*$^-$ mutants, including *alc1*, is temperature sensitive. This effect is amplified in certain RNA polymerase mutants of *E. coli* of the Rif$^{\text{R}}$-2 type[24] and *alc1* will not form plaques at 42 °C on these strains. We think the temperature sensitivity is due to the *alc*$^-$ mutation because (1) a fairly high percentage (~10%) of spontaneous *alc*$^-$ mutants are temperature sensitive, and (2) we have not been able to separate the temperature sensitive and *alc*$^-$ phenotypes by recombination.

Even though we do not know the molecular basis of the temperature sensitivity we have used it to map *alc*$^-$ mutations. First, we isolated the *alc1* mutation by crossing out the amber mutation and the rII deletion which were used in its selection[17]. We then crossed this temperature-sensitive mutant with amber mutants in genes around the T4 map and selected for non-temperature sensitive, *am*$^+$ recombinants. We obtained the lowest recombination frequencies with mutants in the region of gene *31*. To localise the mutation more precisely, we constructed double mutants of the temperature-sensitive mutant and amber mutants in genes in this region. We then crossed these against

Table 1 Map position of *alc*⁻ mutations

Cross Crossover occurring	*am*H21, *alcl* × *am*H39 54 alc	*am*H39, *alcl* × *am*H54 30 alc	*am*N54, *alcl* × *am*M69 31 alc	*am*M69, *alcl* × *am*N54 alc 63
	30	31	63	31
Expected frequency *alc*⁻	~1	~1	~½	~½
Observed frequency	$\frac{50}{56}$	$\frac{57}{65}$	$\frac{43}{112}$	$\frac{56}{97}$

Map order: 54 30 31 *alc* 63

Double mutants *am*, *alc*⁻ were constructed by crossing and screening *am* progeny for temperature sensitivity by spotting on K803 Rif^r–2[23] at 42 °C. These double mutants were then crossed with single amber mutants in the region of gene 31. The *am*⁺ recombinants were spotted on K803 Rif^r–2 to determine their temperature sensitivity. Shown is the fraction of the total *am*⁺ progeny from one plate which are *alc*⁻. The amber mutants used were *am* H21 (gene *54*), *am* N54 (gene *31*) and *am* M69 (gene *63*). The *alc*⁻ mutant is *alc1*, the same mutant used in the RNA polymerase experiment in Fig. 1. *alcl* is also *unf*⁻. We have also analysed other crosses, the results of most of which were also consistent with this map order.

single amber mutants and tested the *am*⁺ progeny for their temperature sensitivity (Table 1). We have shown the map order which is consistent with the data and the crossover occurring in each case. The data support the map order gene *63*-*alc*-gene *31*.

There are four other types of mutations which have been reported to map between genes *63* and *31*. These are *r*III, *cd*, *pset* and *unf* (ref. 11). Of these, *unf* may be related to *alc* since they both might be expected to play a part in host shutoff, *unf* because it is required for the unfolding of the bacterial nucleoid and *alc* because it prevents a type of transcription on cytosine-containing T4 DNA. Thus, we studied the ability of *alc*⁻ mutants to unfold the host nucleoid after infection.

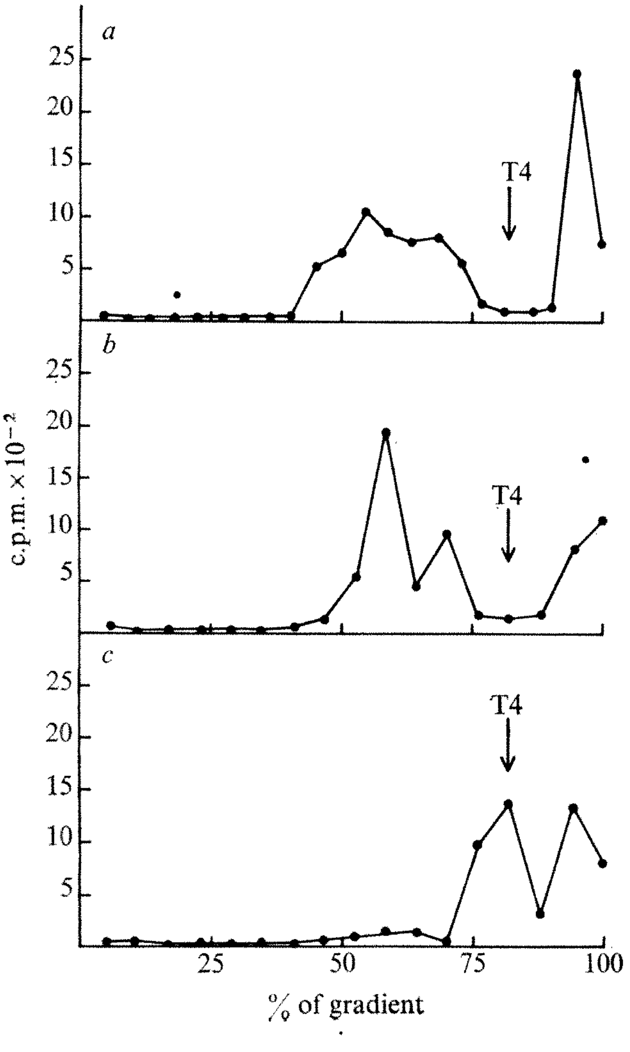

Fig. 2 Sucrose gradients of nucleoids after infection by an *alc*⁻ mutant. *E. coli* B^E (5 ml) growing at 30 °C in M9S were labelled for 1 h before infection with 16 μCi ml^{-1} thymidine (5.5 mCi mg^{-1}). They were infected at a concentration of 4×10^8 ml^{-1} at a multiplicity of infection of 5 in 10 μg ml^{-1} tryptophan. At 5 min after infection, 1.0 ml was poured into a chilled tube containing 0.1 ml of 1 mg ml^{-1} chloramphenicol. The lysis procedure of Snustad *et al.*[11] was used except that the solutions were made up as follows. Solution I: 0.1 m Tris, 6.5 mg sodium azide, 1.0 g sucrose, 0.58 g NaCl, and 9 ml H_2O. Solution II: 1.2 ml Tris, 2.0 ml EDTA, 4 mg lysozyme, and 6.8 ml H_2O. Solution III: 0.1 ml Sarkosyl, 1.16 g NaCl, 0.5 ml EDTA, and 9.0 ml H_2O. Tris was 1 M adjusted to *p*H 8.15 with HCl. EDTA was 0.25 M adjusted to *p*H 7.0 with NaOH. After 5 min for lysis, two additional steps were performed before layering on gradients. 0.1 ml of 20.0 mg ml^{-1} lysozyme dissolved in solution II were added for 10 min and then the lysate was diluted with 2.0 more ml of solution III. Five minutes later the lysate was chilled and 0.15 ml were layered on 4.7 ml 10–30% w/v sucrose gradients made up with 0.5 ml Tris, 0.25 ml EDTA, 0.25 ml Sarkosyl, 5.8 g NaCl, and 10 μl mercaptoethanol per 50 ml. There was a 0.5-ml step at the bottom made from a solution containing 0.1 ml Tris, 6.0 g sucrose, 1.0 g NaCl, 3.0 ml saturated CsCl and 3.0 ml H_2O. They were centrifuged at 4 °C for 25 min at 17,000 r.p.m. in a Spinco SW50L rotor. Fractions were collected from the bottom which is at the left. *a*, Uninfected; *b*, *am*E51, DD2, *alc2* (ref. 17), an *alc*⁻ mutant induced by hydroxylamine and back crossed three times against its parent at a ratio of 1 : 10; and *c*, the *alc*⁺ parent of *alc2*. The arrows show the peak of sedimentation of a T4 phage marker.

alc⁻ Mutants defective in host nucleoid unfolding

In 1971, Stonington and Pettijohn reported a lysis procedure which leaves *E. coli* DNA highly folded, perhaps in a similar state to its intracellular structure[20]. These compact structures, nucleoids, have a high sedimentation rate and contribute little to to the viscosity of lysates. If they are unfolded, however, after T4 infection[12] for example, their S value drops markedly and the lysates are extremely viscous.

To see whether *alc*⁻ mutations affect the T4-induced unfolding of the host nucleoid, we made sucrose gradients of nucleoids from uninfected, *alc*⁻ infected and *alc*⁺ infected cells (Fig. 2). In agreement with others, our nucleoids from uninfected cells sedimented heterogenously with an S value of about 2,000. This S value was largely unchanged by infection with the *alc*⁻ mutant while it dropped to about 1,000S after infection by *alc*⁺ T4. Thus, the *alc*⁻ mutant is defective in host nucleoid unfolding.

We also determined the time course of unfolding by *alc*⁻ mutants by measuring the viscosity of lysates of cells taken at different times after infection. The results for two independent *alc*⁻ mutants and their parent are shown in Fig. 3. Both *alc*⁻ mutants were defective in host nucleoid unfolding compared with the parent. One of them, *alc2*, probably never unfolds the host nucleoid whereas the other, *alc4*, is merely delayed about 1–2 min in unfolding. Some *alc*⁻ mutations do not measurably affect the timing or extent of host nucleoid unfolding.

Are *alc* and *unf* the same gene?

Several considerations support the conclusion that *alc* and *unf* code for the same gene product. A fairly high percentage (> 10%) of *alc*⁻ mutants are also measurably *unf*⁻. Those *alc*⁻ mutants which are not measurably *unf*⁻ are also those which make reduced amounts of late gene prducts when cytosine-containing T4 DNA is made (data not shown). We have also obtained what seem to be partial revertants of the *alc1* mutation (that is, they are less *alc*⁻). These have also become *unf*⁺ to varying degrees. The temperature-sensitive mutation which we have mapped is inseparable from *alc* by recombination and maps in the same region as the *unf* mutation of Snustad *et al.*[11]. So far, those *alc*⁻ mutations which confer the most temperature sensitivity have been the most completely *unf*⁻. The *alc2* mutant used was back-crossed against its parent three times at a ratio of 1:10, selecting the *alc*⁻ phenotype each time; it was still *unf*⁻. All of these observations support the conclusion that *alc* and *unf* are the same gene. However, we have not rigorously excluded two other possibilities. One of these is that *alc* and *unf* are neighbouring genes and those *alc*⁻ mutants which are also *unf*⁻ are deletions spanning these two genes. The existence of partially *unf*⁻ mutants such as *alc4*, however, which is also intermediate in its temperature sensitivity and *alc*⁻ phenotype, argues against the deletion hypothesis. Also, the high frequency of *alc*⁻,-*unf*⁻ mutants has already been given as evidence for point mutants. The other possibility is that *alc*⁻, *unf*⁻ mutants are double point mutants, *alc* and *unf* are adjacent genes, both the *alc*⁻ and *unf*⁻ mutations enhance the multiplication of cytosine-containing T4, and the temperature sensitivity is due to the *unf*⁻ mutation. However, we think these alternative explanations are very unlikely and that *alc* and *unf* are the same gene.

If *alc* and *unf* are the same gene why are not all *alc*⁻ mutations measurably *unf* deficient? The most likely explanation is that T4 mutants which are only partially defective in *alc* function can still multiply with cytosine in their DNA to make a plaque; but are not as temperature sensitive and are scored as *unf*⁺ in our unfolding test.

Implications for host shutoff and *E. coli* nucleoid structure

Although *alc* might be expected to play a part in host shutoff, *alc*⁻ and *unf*⁻ mutants do not affect the timing of shutoff of synthesis of the host proteins one sees on polyacrylamide gels (ref. 11 and our unpublished observations). Since there could be a translational shutoff superimposed on the transcriptional one, however, we looked at the shutoff of host RNA synthesis directly by testing to see whether any RNA synthesised after infection by an *alc*⁻ mutant can hybridise to *E. coli* DNA. The results are shown in Table 2. It is clear that the *alc*⁻ mutation affects the shutoff of the synthesis of at least some *E. coli* transcripts. Thus, selecting T4 mutants which could multiply with cytosine in their DNA has led to the discovery of a host shutoff gene, *alc*.

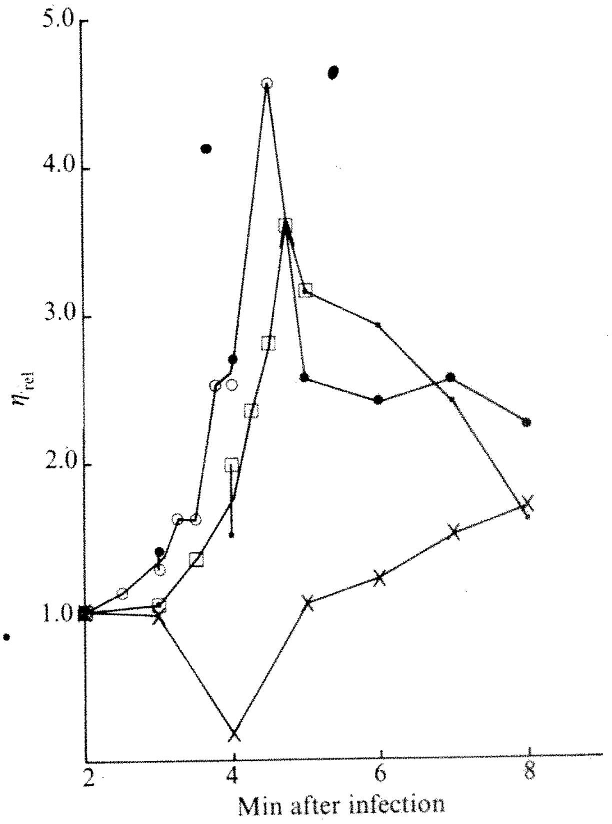

Fig. 3 Time course of unfolding of host nucleoids after infection by *alc*⁻ mutants. Cells were infected and all 5 ml lysed as in Fig. 2. 12 min after solution III was added, the viscosity of the lysate was measured without previous centrifugation by measuring the rate of movement in mg s⁻¹ through a 20-gauge needle 4 cm long. The ratio of this number to that obtained from lysates of cells at 2 min after infection is plotted. Lysates of uninfected cells flowed at close to the same rate as cells collected 2 min after infection and both were only slightly slower than H_2O. The process of measuring the viscosity of all the samples took less than 15 min and the uninfected cell lysates were still not viscous at this time. The closed and open figures of each type represent different experiments. We drew the line through the midpoint when the results were slightly different for the same time after infection. (○), *am*E51, DD2 (ref. 17); □, *am*E51, DD2, *alc4*; ×, *am*E51, DD2, *alc2*.

Table 2 Hybridisation of RNA labelled after infection by an *alc*⁻ mutant to *E. coli* DNA

RNA ^{3}H	Labelling time	c.p.m. Input	c.p.m. Hybridised	% of RNA Hybridised	% of RNA which is *E. coli*
Uninfected	2-min pulse	8,3[illegible]7	1061	12.7	(100)
T4+	4–6	4,736	107	2.26	17.8
	8–10	3,033	31	1.04	8.2
	14–16	2,557	103	4.0	31.5
alc1	4–6	5,048	224	4.4	34.6
	8–10	3,495	196	5.5	43.0
	14–16	2,840	261	9.2	72.5

RNA was pulse labelled at the times indicated in M9 medium at 30 °C with 10 μCi ml⁻¹ ^{3}H-uridine (5 Cimmol⁻¹). The phage were *alc1* which had been crossed against the wild type (T4⁺) to remove the *am*E51 and DD2 mutations[17]. The multiplicity of infection was 10 and tryptophan was added at 10 μg ml⁻¹ before infection. Surviving bacteria were less than 0.1% at 2 min after infection. The RNA was extracted three times with phenol at 60 °C by the Method I of Bolle *et al.*[18], ethanol precipitated, treated with DNase (5 μg ml⁻¹) in 0.1 M Tris-Cl *p*H 8.0, 0.01 M $MgCl_2$ for 15 min at 37 °C, extracted twice and reprecipitated with ethanol. Hybridisation was with 20 μg ml⁻¹ *E. coli* DNA[30] for 40 h at 60 °C in 2SSC. The hybrids were treated with RNase (12 μg ml⁻¹ RNase A at 37 °C for 15 min), collected on presoaked Millipore filters, and washed with 0.5 M KCl, 0.01 M Tris, *p*H 7.5. The specific activities of the RNAs were from top down, 10.6, 6.76, 4.0, 3.32, 7.8, 5.85, and 4.27 c.p.m. ng. The percentage of RNA which is *E. coli* was determined by dividing the percentage of input counts which hybridised by the percentage of the uninfected labelled RNA which hybridised. This number is affected by changes in the relative concentrations of *E. coli* RNA species so should only be considered qualitative. It is perhaps surprising that *E. coli* transcription can continue so late into infection when the host DNA is being degraded. This RNA is presumably being made on fragmented DNA.

If *alc*$^-$ mutants are defective in the shutoff of host transcription, why are they normal in the shutoff of the host proteins one sees on gels? One possibility is that there is a translational shutoff which is independent of transcriptional shutoff, and evidence has been presented for such a translational shutoff by T4 (refs 27 and 28).

Host RNA is still being made after infection even by *alc*$^+$ phage, as also observed by Kennel[27]. In fact, at late times the percentage of RNA synthesis attributable to the host apparently increases (see Table 2). Either *alc* is inefficient in its shutoff of host transcription or it is only responsible for shutting off a subset of the host RNA synthesis. Accordingly, we are undertaking experiments to determine whether all types of host transcription are shut off equally by *alc*.

In conclusion, we have presented evidence that an RNA polymerase subunit can cause the host nucleoid to unfold. Since mutants which lack the function of this subunit allow increased transcription of the host genome after infection, perhaps the *alc* gene product prevents the synthesis of RNA which holds the nucleoid together. In this connection, it has been reported that treating cells with rifampin causes the unfolding of the nucleoid[26]. Other types of experiment also suggest that RNA is important in maintaining the highly folded state of the host nucleoid since RNase treatment can cause it to unfold[20]. According to one model, then, the unfolding of the host chromosome is merely a consequence of shutting off host transcription by *alc*. However, another possibility should be considered: the unfolding of the host chromosome by *alc* may cause host transcription to cease. If so, perhaps T4 does not make RNA on cytosine-containing T4 DNA because *alc* causes it to "unfold itself". It should be possible to design experiments to test these, and other, hypotheses. Because of this, the T4 *alc* gene product promises to be an important tool in the study of the modes and mechanism of prokaryotic transcription. It also promises to be useful in the study of the roles of RNA and RNA polymerase in maintaining the structure of the prokaryotic chromosome.

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letters to nature

Evidence from optical polarisation for a galactic-scale uniform magnetic field in M104

LINEAR polarisation of light has been observed in several galaxies[1–4], but the only previous detailed polarisation pattern has been mapped in M82[2,3,5,6] from which information about the luminous and physical structure of the galaxy has been deduced[7]. Clearly, detailed knowledge of the polarisation pattern in a nebula is a powerful tool for further understanding of the nebula. In this letter we present a map of the linear optical polarisation of the Sombrero Galaxy (M104) giving the first clear evidence for the existence of a magnetic field which maintains the same projected direction over an estimated volume of some 10^3 kpc^3.

The observations were made on the 1-m telescope of the Wise Observatory, Israel during the period May 20–24, 1976 using the B waveband of the UBV system. The technique used has already been described[8] and uses the Durham polarimeter and the Royal Greenwich Observatory 4-cm electronographic camera to record the analysed light on nuclear emulsion. The electronographic plates have been digitised on the PDS machine at the Royal Greenwich Observatory with a bin size of 1.4 × 1.4 arc s. The polarisation measurements have been determined at each point over an integration area of 7 × 7 arc s (that is, over 25 digitisation bins). Measurements have been made at 1,000 points within the galaxy. Calibration checks on the performance of the polarimeter have been made using standard polarised stars as references[8]. It has not been necessary to make instrumental corrections.

The resulting polarisation map is shown in Fig. 1. Our technique eliminates any non-uniformity of the electronographic emulsion as we make two independent measurements at each point and these agree in all cases on this map. Each measurement is indicated by a line in the direction of the E-vector, proportional in length to the degree of polarisation, and centred on the point observed. The map is superimposed on a photograph of M104. The polarisation in the dark lane has an average value of $2.2 \pm 0.5\%$ and position angle of $86° \pm 5°$. A previous single measurement in this area by Appenzeller[4] is in agreement with our values if we integrate over a similar area to that used by Appenzeller.

The main features of the polarisation map are: (1) An almost uniform polarisation parallel to and along the central dark lane spanning an area at least ~ 100 × 30 arc s, which at a distance of 12 Mpc (ref. 9) is equivalent to 6 × 1.6 kpc. The orientation of the polarisation is also maintained over the brighter part of the lower optical lens. (2) In the upper lens the electric vectors are perpendicular to the dark lane. The transition from parallel to perpendicular orientation of the electric vectors is abrupt.

In Fig. 2 we show the intensity (I) and polarised intensity (P) as functions of position along a scan line perpendicular to the dark lane and through the nuclear region. We note several

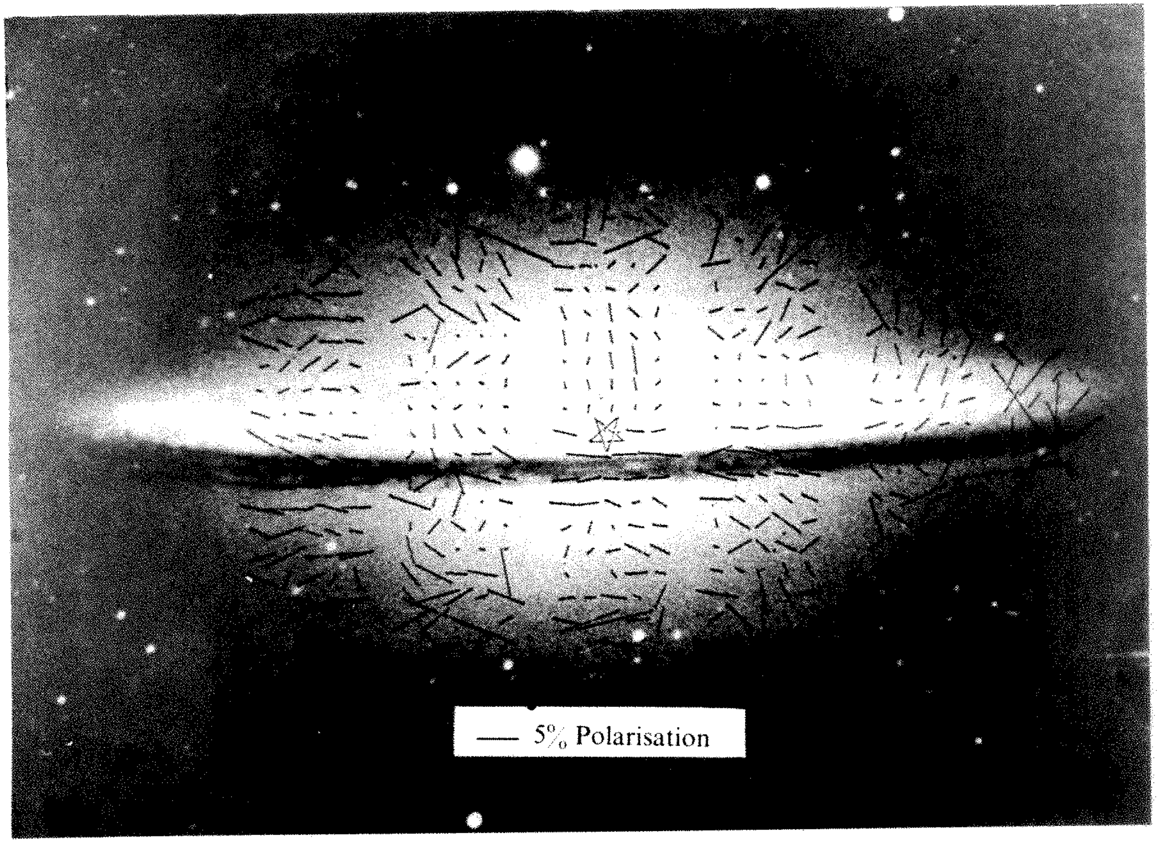


Fig. 1 The linear optical polarisation of M104 in the B waveband. *Represents position of nucleus.

features of the graphs. (1) The centre of the dark lane is readily apparent in the graph of *I*; it is at the pronounced dip and below the maximum of *I*. (2) The maxima of *I* and *P* are not coincident; the former maximises (I_{max}) at 21 arc s N of the dark lane and the latter (P_{max}) at 14 arc s N. (3) While neither *P* nor *I* is symmetrical about its maximum, *P* is more symmetrical. The change from parallel to perpendicular alignment of the **E**-vectors occurs between the maxima of *P* and *I*.

Finally we have integrated over the whole electronographic plate and estimate that the apparent nucleus contributes approximately 20% to the total luminosity.

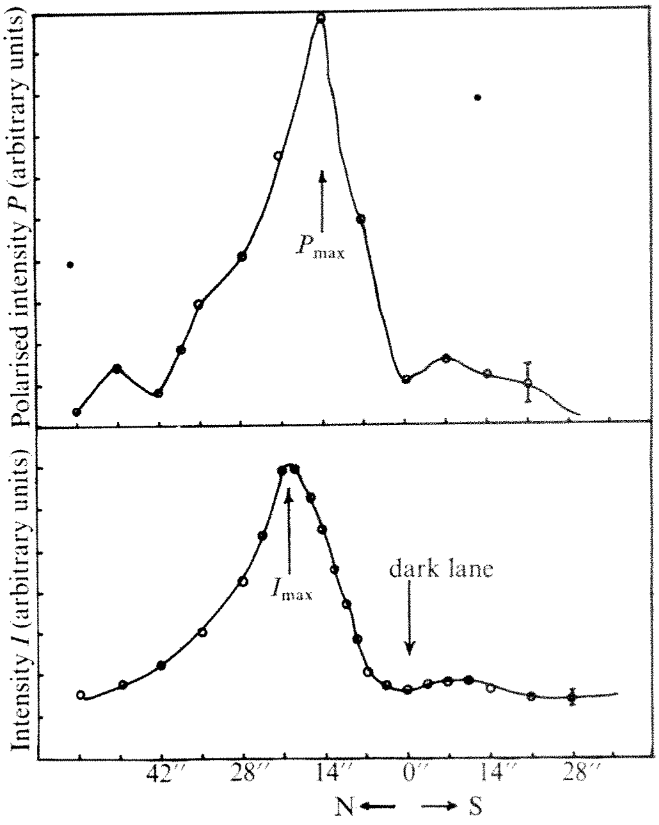
There is little doubt that the observed polarisation pattern is due to the scattering and extinction of light by statistically aligned dust grains. Synchrotron emission is eliminated as M104 is neither a radio[10] nor an X-ray source[11]. Rayleigh scattering can also be eliminated as the illumination of an optically thin Rayleigh atmosphere by a nucleus plus disk produces a distinctive centro-symmetrical pattern[7] quite unlike that observed.

The alignment of the dust grains is most probably due to magnetic effects as proposed by Davis and Greenstein[12]. The alignment in our Galaxy has been the subject of much discussion; radiative transfer of angular momentum by optical[13] and X-ray[14] photons and gas streaming[15] are alternative processes to the Davis–Greenstein effect for dust alignment. However, it is difficult to see how the alternatives could be effective in the case of M104 as the optical photons would produce alignment in the wrong sense to that observed; for gas streaming the alignment would be slowly changing with no polarisation observed at the outer edge of the disk: this again is not observed. Thus unless some heretofore unexpected mechanism is at work the grains must be magnetically aligned.

The change in orientation of the polarisation vectors from perpendicular to parallel to the dark lane is probably the result of increasing dust density towards the plane of the galaxy and a corresponding change of polarisation by scattering to extinction. This then implies that the magnetic field maintains its orientation not only for another 2 kpc above the galactic plane but in depth as well.

The condition for the transition in orientation is that the polarised intensity from scattering (P_s) of disk light must exceed the polarised intensity from extinction (P_e) of halo starlight beyond 21 arc s above the dark lane. Since light scattered by aligned dust grains[16] (submicrometre prolate spheroids) is polarised by some 50% (for scattering optical

Fig. 2 Graphs of the intensity and polarised intensity for a scan in the N–S direction through the central region of M104.



depth $\tau_s < 1$) we have $P_s \approx 0.5\ \tau_s L_D/4\pi h^2$ where L_D is the galactic disk luminosity (nucleus included) and h is the height above the disk. P_e is given by $P_e \approx 3.10^{-2}\ e(-\tau_e) n_* L_* Z/4\pi$ where n_* and L_* are the density and luminosity of halo stars, Z is the line of sight distance through the halo, and τ_e is the optical depth for extinction. A polarisation of 3% is assumed for extinction. For $P_s > P_e$, one has

$$\tau_s L_D > 0.1(1-\tau_e)L_H$$

where $L_H \approx n_* h^2 Z L_*$ is the total luminosity of halo stars in the volume $h \times h \times Z$. Since $L_D > L_H$, this condition is easily satisfied far above the disk.

As h decreases however, the densities of both dust and stars increase, and stars which were illuminating dust above the galaxy begin to contribute to L_H, with a corresponding decrease in L_D, so that extinction begins to dominate. For low values of τ_s, small changes in n_* and $\tau_e \approx \tau_s$ can change the character of the polarised light completely, and this transition is what one observes 21 arc s N of the dark lane's centre. Evidently, the condition is not satisfied in the southern hemisphere of the galaxy, and extinction predominates.

From the fact that the I_{max} and P_{max} are not coincident, we infer that the brightest parts of the nucleus and disk are obscured. In fact, it seems that the (obscured) brightest part of the galaxy is between 7 arc s and 14 arc s N of the dark lane centre, near where P_{max} occurs. Here the optical depth τ_e must be larger than one, so that over some 14 arc s, τ_e varies by about two or three orders of magnitude. This implies a scale height of 100–200 pc for the dust, which is a typical galactic scale.

The fact that scattering from aligned grains is occurring makes M104 a likely candidate for observation of circular polarisation. If recent calculations[16] are applicable, then a circular polarisation of some 15% is introduced in the scattered light, which means we expect some 0.7% circular polarisation to be observable after dilution by halo starlight. The maximum degree of circular polarisation should be in the halo, but since the optical depth is large in the dark lane, the maximum absolute amount may be observed there. An observation of circular polarisation would tend to confirm our interpretation of the linear polarisation.

We have presented here our preliminary results, and fuller analysis and detailed models will soon follow. It seems, however, that we are seeing the most extensive homogeneous magnetic field observed so far and it is tempting to speculate that it may be primordial in origin.

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Verification by Viking Landers of earlier radio occulation measurements of surface atmospheric pressure on Mars

UNTIL this year, the most accurate means of studying the atmospheric pressure at the surface of Mars were provided by remote sensing from fly-by or orbiting spacecraft. Radio occultation, which involves observations on Earth of the effects of refraction on the radio link from the spacecraft in the Martian atmosphere, has been used repeatedly to measure the pressures and temperatures in the atmosphere of Mars from the US Mariner[1] and Soviet Mars[2] spacecraft. By far the largest number of these measurements, some 260, was obtained with the Mariner 9 orbiter in 1972–73[3].

The landing of Viking 1 in Chryse Planitia on July 20, 1976 provided the first opportunity to obtain measurements of atmospheric pressure directly from the surface of Mars. In the interest of comparing and verifying radio occultation results relative to the Viking *in situ* measurements, a computation was carried out to predict the atmospheric pressure at the landing site before the landing itself.

Because no radio occultation measurements existed near the Viking 1 site (22.3°N, 48.0°W), it was necessary to have a means of obtaining the relative altitude between occultation points and the Viking 1 site. This link was provided by Earth-based planetary radar data taken in 1967 (ref. 4), covering Martian latitudes from 19°N to 24°N. Four Mariner 9 occultation points lying within these latitudes were used to compute the distance from the centre of Mars (radius) of the zero-elevation reference level of the radar elevation profile (ref. 4, Fig. 4). These were points 77*N* (20.8°N, 337.7°W), 78*N* (24.1°N, 154.7°W), 418*N* (24.9°N, 180.9°W), and 420*N* (22.0°N, 172.4°W). The number designating the occultation measurement refers to the number of the revolution of the orbiter about Mars, and *N* stands for a measurement taken during entry into occultation. Combining the radii measured at those four points with the elevations indicated by the radar profile yielded a zero-altitude radius of $r_0 = 3389.54 \pm 1.5$ km. The uncertainty was estimated on the basis of uncertainties in both the radar and occultation measurements.

The predicted pressure at the Viking 1 site can then be estimated either with reference to a 6.1-mbar areoid computed from the gravity field of Mars and Mariner 9 occultation data[5] or by direct comparison with occultation points consistent with the areoid. The results are displayed in Table 1.

The quantity Δh is the altitude difference between the Viking 1 landing site and the 6.1 mbar areoid in the first instance, and the radii measured at the respective occultation points in the second two. These are then used, assuming a 12-km scale height (corresponding to the expected atmospheric temperature at the time of Viking 1 landing) to extrapolate the pressures shown in the column labelled 'source' to the pressure at the Viking 1 site, which is given in Table 1, column 3.

These estimates of pressure are valid for the season of Mariner 9 measurements. Because of the interaction of the atmosphere of Mars with the polar caps, however, the global atmospheric pressure is expected to vary over a Martian year by as much as 1.7 mbar[6]. Although much uncertainty is attached to these estimates of seasonal variation, which will be definitely established by Viking measurements, it was estimated that the pressure should be lower by about 0.2 mbar at Viking 1 arrival relative to Mariner 9. This correction was made, and the corrected figures appear in Table 1, column 4. Finally, an average of the three determinations was taken, yielding a prediction of 7.6±1.1 mbar.

The mean diurnal atmospheric pressure actually measured by Viking 1 was 7.65 mbar by the meteorology experiment[7] and 7.3 mbar by the entry science experiment[8].

The near exact correspondence of the measured and predicted pressure is of course fortuitous in view of the 1.1-mbar uncertainty attached to the prediction. Nevertheless, it indicates that the radio occultation results from Mariner 9 closely correspond to the actual surface pressure on Mars, which should enhance the credibility of the radio occultation method and of results obtained from planets and satellites whose atmospheres have not been directly probed.

Table 1 Estimates of pressure

Source	Altitude difference Δh (km)	Pressure at Viking 1 site (mbar) Mariner 9 season	Viking 1 season
Areoid $p=6.1$	-2.93 ± 1.5	7.8 ± 1.0	7.6 ± 1.0
Occ. pt 420N $p=6.7\pm0.5$	-2.46 ± 1.5	7.5 ± 1.1	7.3 ± 1.1
Occ. pt 78N $p=7.65\pm0.5$	-0.67 ± 1.5	8.1 ± 1.2	7.9 ± 1.2
Average, corrected for season			7.6 ± 1.1

A more cursory prediction was made for the Viking 2 landing site (48.0°N, 225.7°W). In the absence of planetary radar data, a Mars contour map compiled from Mariner 9 data[9] was used to obtain altitude differences, and less reliable extended mission data were used. After correction for seasonal variations, a pressure of 6.7±1.4 mbar was predicted. The actual pressure as measured by the meteorology experiment (unpublished data) was 7.72 mbar, within the uncertainty bounds of the prediction.

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Search for superheavy elements in monazite by Rutherford backscattering

EVIDENCE that superheavy elements with $Z=116$, 124 and 126 may exist in monazite inclusions associated with giant haloes in biotite mica has recently been reported[1]. As there is no evident reason why such elements should not also exist elsewhere, it would seem fruitful to search for superheavy elements in monazite inclusions not associated with giant haloes, or even in bulk monazite, although their concentrations in such environments may be considerably lower than in giant halo inclusions.

In this letter, we report the results of such a search in samples of bulk monazite by a technique capable of detecting concentrations of order 1 p.p.m. A beam of ^{32}S ions is Rutherford scattered[2] backwards from the sample and the energies of the scattered ions are measured. The energetics of the scattering process are described by classical mechanics; at exactly 180°, for example, the energy of a particle of mass m and incident energy E_i scattered by a nucleus of mass M is just $E_{sc}=((M-m)/(M+m))^2E_i$ for $M>m$; if $M<m$ no scattered events will be observed and this is the case for all angles greater than 90°. As the target mass M increases, so also does the energy of the scattered particle. With the incident energy known, a measurement of the scattered energy can therefore be used to deduce the mass of the target nucleus.

We have used beams of 42 MeV ^{32}S ions from the Oxford Tandem van de Graaff accelerator to bombard targets of monazite sand obtained from the mineralogical collection of the University Museum and originating from India. The sand was finely powdered and glued on to aluminium backings; the resulting monazite targets were sufficiently thick (several mg cm^{-2}) to stop the beam. No backscattered events from the aluminium or from the constituents of the organic glue can occur because these elements are lighter than sulphur. The scattered sulphur ions were detected at a mean angle of 165°, the largest angle at which our detectors could be placed without interfering with the incident beam. Two thick silicon surface barrier detectors (50 μm and 75 μm) were used on opposite sides of the beam. The primary constituents of monazite, essentially (Ce, La, Y, Th) PO_4, which can scatter sulphur ions into the backward hemisphere are the lanthanides (mainly Ce and La), Th and U. The phosphorus and oxygen are again lighter than sulphur and thus will not contribute. In the Table, we give the maximum energies of the backscattered sulphur ions from these and a possible superheavy constituent, under the experimental conditions mentioned above.

The energy spectrum of backscattered sulphur ions obtained from a 10 h exposure with one of the detectors is shown in Fig. 1. (A similar spectrum was obtained with the other detector.) Notice that the vertical scale is logarithmic, but with zero included; energy is plotted along the horizontal axis. The sharply cut off edges correspond to the maximum scattered energy from each of the elements indicated; i.e. from the target surface. The plateaux extending down to zero energy are due to the fact that the incident or scattered ion beam can lose all or part of its energy in the target.

If elements with $A>238$ are present, then scattering from such nuclei would give rise to events above the Th+U edge (Table 1). Figure 1 shows the position of the edge corresponding to $A=354$ which is a predicted mass number for the most stable isotope of element 126 (ref. 3). However, such events can also arise from 'pile-up' caused by the simultaneous detection of two (or more) pulses of lower energy, and this limits the sensitivity of the technique. In

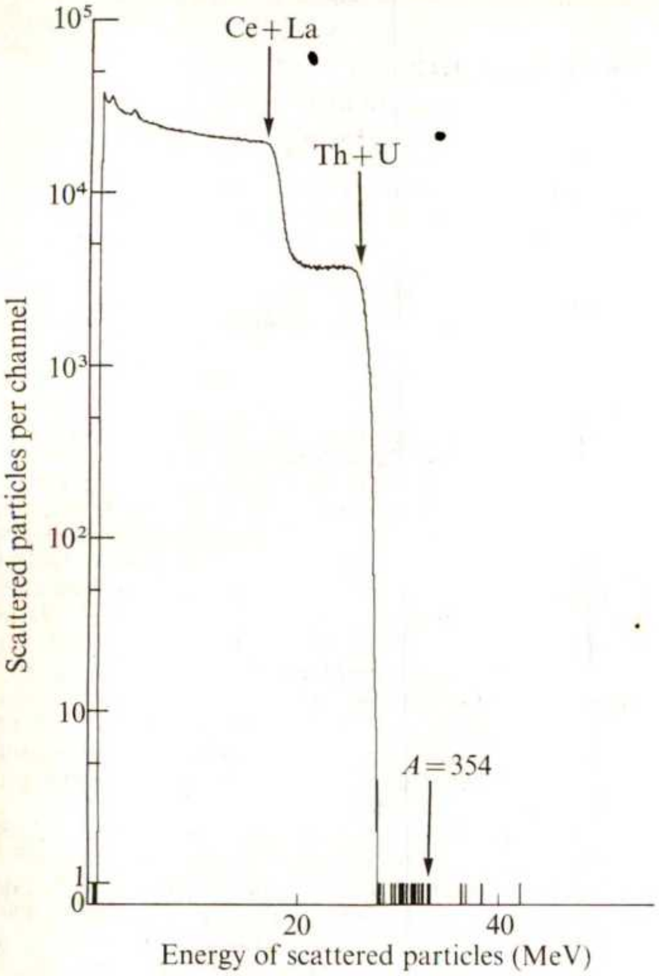

Fig. 1 Energy spectrum of ^{32}S ions of incident energy 42 MeV scattered from a target of powdered monazite sand observed at an angle of 165°. The edges correspond to scattering from the various constituents of the target (see text). An energy of ~24.5 MeV corresponds to channel 500 and there are an average of < 0.2 counts per channel in the region between the Th+U edge and the calculated position of the edge for $A = 354$.

the spectrum shown, there are 21 counts above the Th+U edge. Even under the improbable occurrence that these events were all due to scattering from superheavy nuclei, the level of the rare earths in Fig. 1 implies an upper limit of 2 atomic p.p.m. of superheavy nuclei of mass 354 (and approximate charge 126) relative to the concentration of the rare earths (mainly Ce and La) in our sample, where the Z^2 dependence of the Rutherford scattering cross section has been taken into account. (An analysis of the spectrum obtained with the other detector gave an upper limit of 1 atomic p.p.m.).

By using heavier projectiles than sulphur (for example, iodine or uranium) this limit might be reduced by a significant factor. A uranium beam is particularly attractive as only scattering from target nuclei with $A>238$ can contribute to the spectrum of scattered particles observed at angles greater than 90°. Such beams may well permit the detection of superheavy nuclei, if they exist, at very low concentrations indeed.

Table 1 Calculated energy of ^{32}S ions, incident energy 42 MeV, with observation at 165° after scattering from various heavy constituents of monazite

Element	Mass number	Energy (MeV)
Ce	140	16.81
Th	232	24.33
U	238	24.67
(126)	354	29.40

A value is included for a possible superheavy element with mass 354.

We thank Dr F. B. Atkins, Curator of the Mineralogical Collections of the University Museum for the provision of the monazite and Dr M. A. Grace and Dr H. J. Rose for valuable suggestions.

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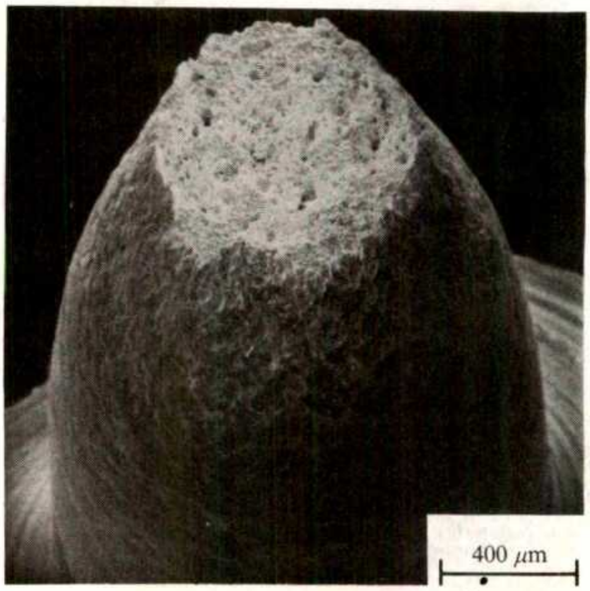
Cadmium metal embrittlement of Zircaloy-2

EFFECTS of chemical environments upon the mechanical properties of zirconium alloys are of great interest because such alloys are used to contain fissionable fuel in water-cooled nuclear reactors. Particular interest attaches to stress corrosion cracking or liquid metal embrittlement because both of these phenomena are known to produce non-ductile fractures in otherwise ductile materials. Rosenbaum *et al.* reported iodine stress corrosion cracking of Zircaloy-2 some years ago[1,2], and this phenomenon has been extensively investigated by others[3–5]. Zircaloy-2 is an alloy of zirconium containing by weight about 1.5% tin, 0.15% iron, 0.1% chromium, 0.05% nickel and 0.12% oxygen. Cox has reported embrittlement of Zircaloy by mercury or liquid caesium at room temperature[3]. However,

Fig. 1 Scanning electron micrograph of a fracture surface of Zircaloy-2 broken in caesium at 300 °C; elongation rate 2×10^{-3} inch min^{-1}, sample guage length 0.13 inch.



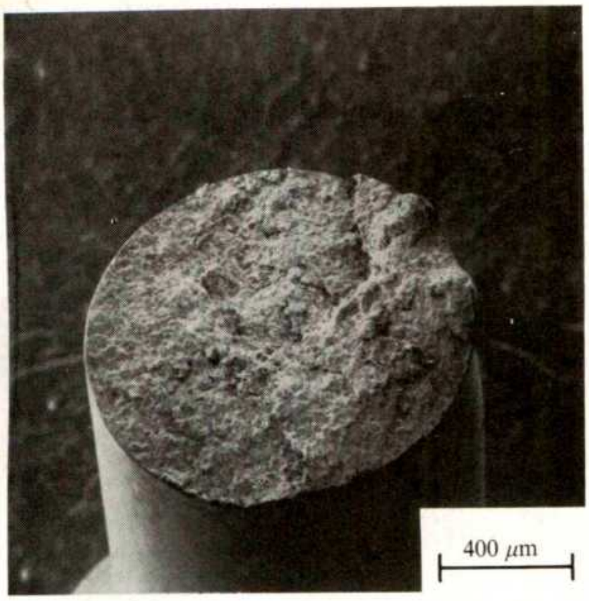


Fig. 2 Scanning electron micrograph of a fracture surface of Zircaloy-2 broken in caesium saturated with cadmium at 300 °C; other conditions as in Fig. 1.

the phenomenon of liquid metal embrittlement is known to be favoured by low temperatures[6], and Zircaloy nuclear fuel cladding operates in the vicinity of 300 °C.

It is the purpose of this note to report the observation of embrittlement of Zircaloy-2 in the temperature region of 300-340 °C by cadmium. Embrittlement occurred in the presence of solid cadmium at 300 °C, liquid cadmium at 340 °C, or cadmium dissolved in liquid caesium at either temperature. On the other hand, caesium alone did not embrittle Zircaloy-2 over this temperature range. Embrittlement of titanium alloys by solid or liquid cadmium has been reported by others[7–9].

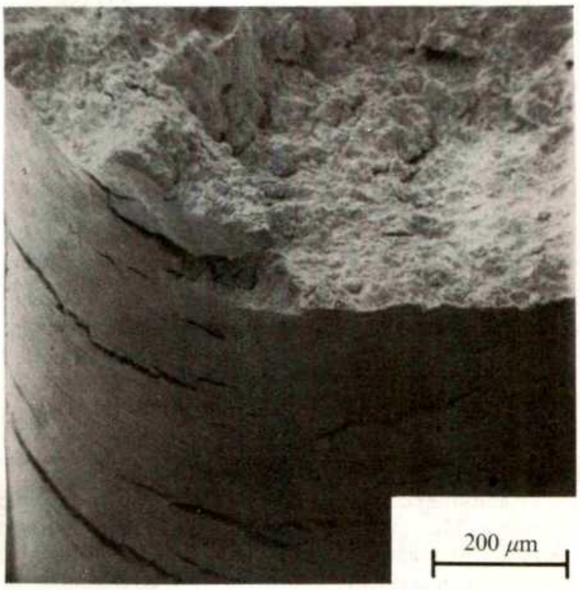


Fig. 3 A portion of the sample of Fig. 2 showing side cracks.

Figures 1, 2, and 3 show fracture surfaces of samples broken in caesium (Fig. 1) or caesium saturated with cadmium (Figs 2, 3) at 300 ± 10 °C. The fracture in caesium occurred with a reduction of area of about 75% and shows a typical ductile morphology similar to that observed in an inert argon environment. The fracture in caesium plus cadmium occurred with substantially zero reduction of area, and the fracture surface seems non-ductile. A portion of that surface at higher magnification is shown in Fig. 4. The mode of fracture is clearly transgranular with quasi-cleavage faces. It is, in fact, similar in appearance to fracture surfaces obtained by breaking Zircaloy-2 in iodine vapour at 300 °C (ref. 3).

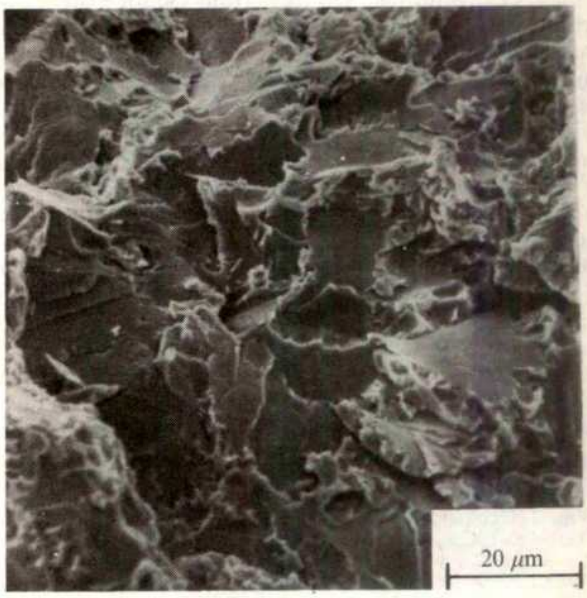


Fig. 4 A portion of the fracture surface of Fig. 2 at high magnification.

The tensile samples of Figs 1–4 were smooth-bar samples cut in the thickness direction from cross-rolled Zircaloy-2 plate. The plate was prepared according to a mechanical schedule described by D. Lee[10] to provide a high degree of orientation of the *c* (0001) axis in the thickness direction. Cracks caused by cadmium seem to occur along or near the basal plane perpendicular to the *c* axis and the applied stress axis, as Figs 2 and 3 show.

These results seem to be the first observation of cadmium embrittlement of Zircaloy-2. I am investigating the phenomenon further; the experimental techniques and further results will be reported in the near future.

I thank Morris H. Morgan III for experimental assistance, D. Lee for samples and advice on cross-rolled Zircaloy, and A. W. Urquhart for discussions.

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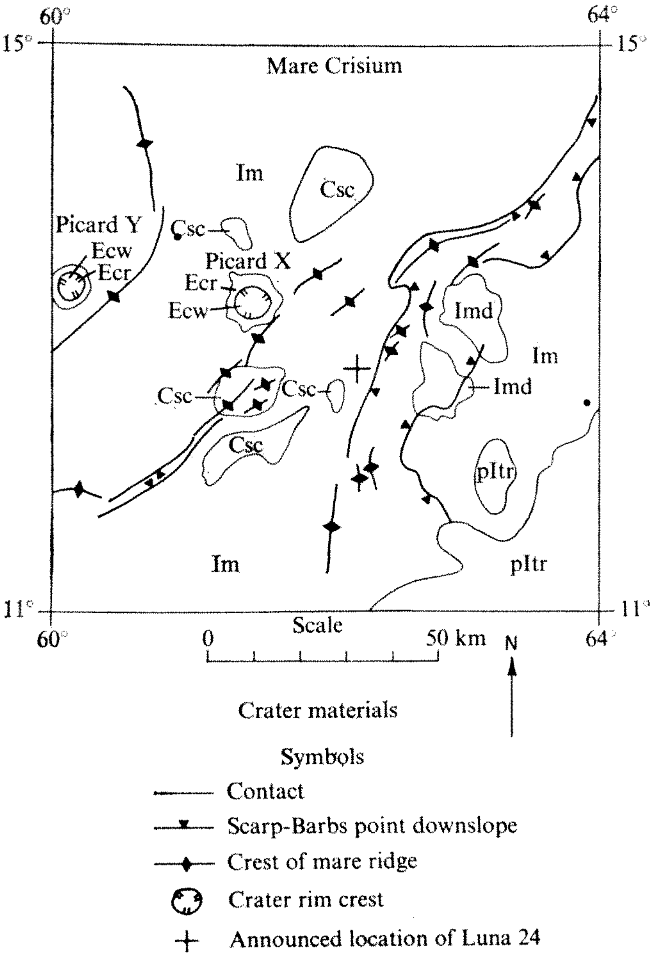
Age of Luna 24 mare basalts based on crater studies

THE recent return of regolith material from Mare Crisium by Luna 24 has provided an opportunity to test our knowledge of lunar impact-flux history. This can be done by comparing crater density data for the landing site with a calibrated impact flux curve to estimate the absolute age of the sample before chemical analysis.

Luna 24 returned samples from south-eastern Mare Crisium (12.7°N, 62.2°SE). The area around the landing site was mapped (Fig. 1) in detail from Apollo panoramic photographs. Obvious swarms of secondaries and endogenic craters were excluded from the crater counts and only one unit was counted at a time. This was to ensure that only the crater (production) population was counted for a single unit. Two major mare units occur in the mapped area; Imbrian mare (Im) on which Luna 24 landed, and dark (low albedo) Imbrian mare (Imd). An additional mare unit may be present just north of the mapped area according to the data of Neukum, König, and Arkani-Hamed[1]. Crater counts were made in two areas, one containing 2,920 km^2 (120 craters counted) of unit Im (including the landing site) and the other 256 km^2 (37 craters counted) of Imd, using Apollo panoramic and metric photographs. Diameter-cumulative frequency curves were constructed from these crater counts. These curves are shown in Fig. 2. C_s (ref. 2), the intersection of Trask's[3] steady-state curve and the extension of the approximately −3 slope crater production curve, is also shown in Fig. 2 for each of the three major mare units in and around the Lunar 24 landing site and the six Apollo landing sites[1-7]. The location of C_s values (which are a measure of impact flux) shows that the Luna 24 landing site is more densely cratered than the Apollo 12 and 15 sites, and less cratered than the Apollo 11, 17, 16 and 14 sites. C_s values for the Apollo landing sites can be compared with Apollo radiometric ages[7-11] to establish the lunar impact-flux history. This curve is shown in Fig. 3 and can be used to estimate the age of the Luna 24 site which is about 3.5 Gyr with a probable error of ±0.1 Gyr. Since the returned sample is of regolithic materials it could also contain basalt fragments which have been transported into the area by impact from the other nearby units. Although this is unlikely, there may be basaltic fragments from the other two units included in the Luna 24 core that are about 3.75±0.1 Gyr (Imd) and 2.5±0.3 Gyr (northern unit).

Fig. 1 Geological map of the Luna 24 landing site. Crater Materials: Csc, Copernican secondary craters; Ecw, Eratosthenian crater wall; Ecr, Eratosthenian crater rim (hummocky facies). Other materials: Im, Imbrian mare; Imd, Imbrian mare dark; pltr, pre-Imbrian terra rugged.



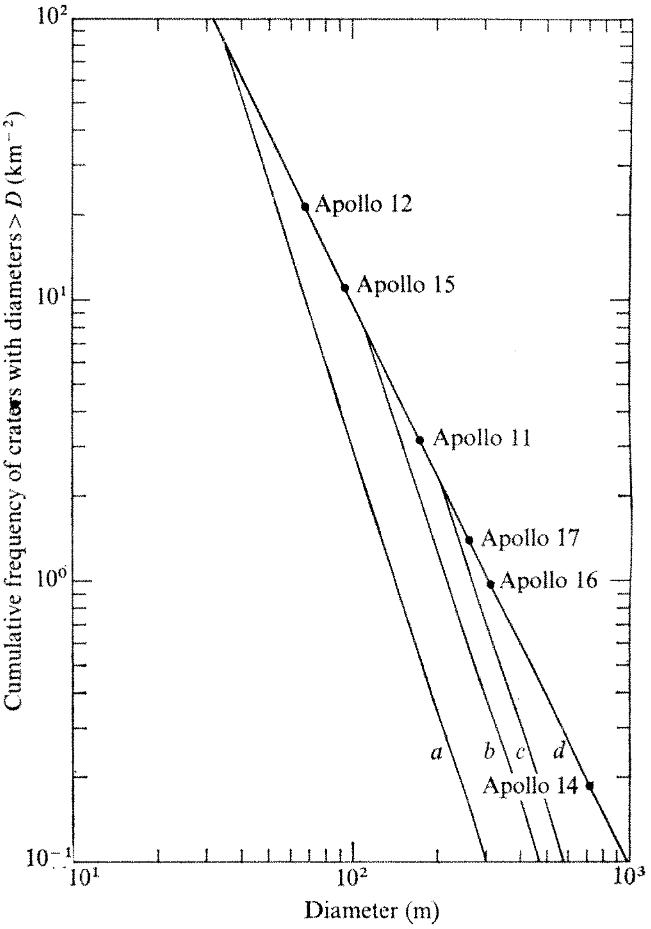


Fig. 2 Cumulative size frequency of craters on the three major mare units (Im, Imd, and the northern unit) in the vicinity of the Luna 24 landing site. The curves are extended to the steady-state curve of Trask's[1] to obtain the C_s values for each curve. C_s values for each Apollo landing site are also included for comparison. *a*, Luna 24 northern unit; *b*, Luna 24 landing site (Im); *c*, Luna 24 (Imd); *d*, $N = 10^{1.9}D^{-2}$.

Additionally, information about the geochemistry of the Luna 24 basalts can be determined from spectral reflectance measurements. Spectral reflectance data[13-15] indicate that most of southern Mare Crisium (including the Luna 24 landing site) is 'red' low-titanium basalt similar to that at

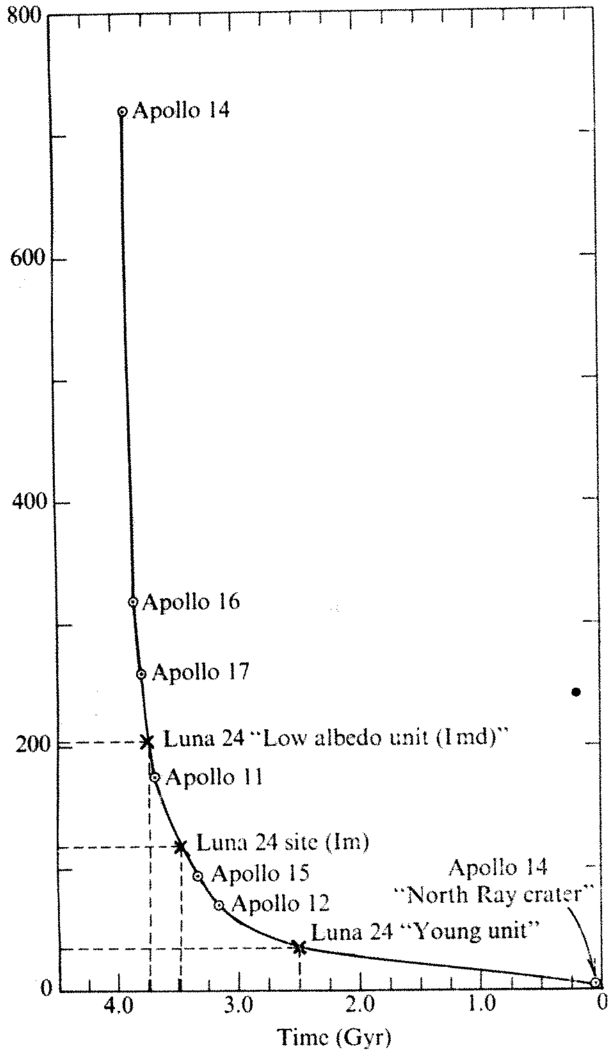


Fig. 3 Comparison of C_s values (a measure of impact flux and relative age) and crystallisation ages of Apollo samples. The estimated ages of the three units that may have been sampled by Luna 24 are included.

the Apollo 15 site. Therefore, based on crater frequency data and spectral reflectance data, we suggest that the mare basalts sampled by Luna 24 are similar in age and composition to those sampled by Apollo 15.

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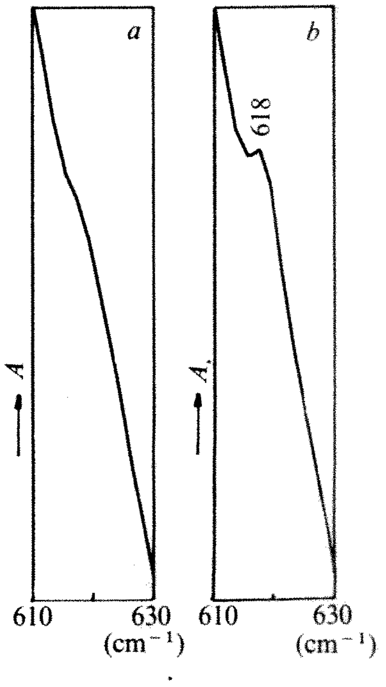
Polymorphic transition in silica glass

THE short range order in glasses is now more or less generally accepted to have a dimension of 20 Å or slightly more[1,2]. In the case of silica glass we have established[3], on the strength of infrared absorption bands, that those regions (clusters, substructures) have the high-cristobalite structure, which is metastable at room temperature and normal pressure. It has been possible to relax a polymorphic transformation in pure silica glass into the stable low temperature form of cristobalite by heating a powdered sample at 483 K for 27 h (ref. 3). Heating relaxed the existing strains in the clusters by adjusting their structure to the low temperature conditions. A report by Wagstaff[4] suggested another possible way of provoking the transition—by increasing the strains in the high-cristobalite clusters in silica glass by cooling it to liquid helium temperature.

Using the attenuated total reflectance (ATR) technique in infrared spectroscopy, we have proved that such a transformation takes place in experimental conditions, after a powdered sample of pure silica glass has been kept for several days at liquid helium temperature. No transition was observed in a piece of this glass kept in the same conditions, which agrees with the result of the heating experiments. The low-cristobalite form of silica shows a characteristic absorption band (618 cm^{-1}) which is absent in the regular high-cristobalite form[3]. This band provides an easy way of observing the transition process. Using powdered mixtures of pure silica glass and known quantities of crystalline low-cristobalite for an approximate calibration, we found about 1% conversion by weight of glass. This is a rather small amount of new phase compared with nearly 100% conversion reached in the heating experiments (but only in some of them). Nevertheless, we have obtained further evidence to confirm the possibility of provoking a

Fig. 1 ATR absorbance plots. *a*, Powdered silica glass before cooling at liquid helium temperature; *b*, the same sample after cooling.



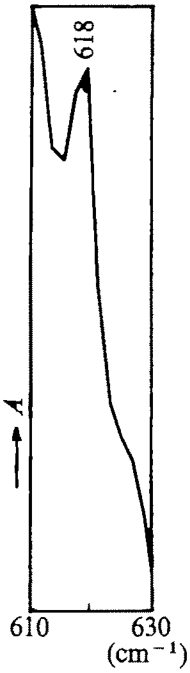


Fig. 2 ATR absorbance plot: computed ratio of Fig. 1*a* and *b* curves. All figures were drawn directly by the Digilab FTS-14 instrument.

polymorphic transition in X-ray amorphous substances, such as silica glass. Independent confirmation comes from the very sensitive thermal expansion observations on silica glass of C. F. Drake, referred to by C. H. Goodman[5].

We are also studying the heat effects of the transition. Preliminary experiments with very sensitive differential scanning calorimetry equipment also unequivocally point to the presence of partial polymorphic transition in silica glass at 542K. This work is still in progress. Thus evidence is accumulating to suggest that polymorphic transitions occur in regions as small as about 20 Å. This, at least in the case of vitreous silica, has also helped to establish firmly the structure of clusters, as polymorphic transitions are strictly characteristic for each form of crystalline silica.

The Digilab FTS-14 Fourier transform spectroscopic instrument was used in this work and appropriate computer programs were used to compare spectra obtained before and after cooling the same sample of glass.

We thank Dr Miroslaw Handke for help with the ATR measurements and Mr Edward Görlich Jr for the opportunity to do the liquid helium experiments.

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Thermoluminescent dating of Lake Mungo geomagnetic polarity excursion

ARCHAEOMAGNETIC studies made by Barbetti and McElhinny[1,2] on prehistoric aboriginal fireplaces at Lake Mungo, Australia, indicate the occurrence of a polarity excursion about 30,000 years ago during which the geomagnetic field intensity increased to three times its present-day value; this main excursion was followed by a second one during which the intensity decreased to about one third of the present day value. This note reports thermoluminescent (TL) dating measurements that were carried out on fireplace material at the request of Dr M. F. Barbetti. Comparison of the TL dating with the radiocarbon ages[3,4] is of particular interest because geomagnetic intensity variations may be the dominant cause of long term distortion of the radiocarbon time scale[5–7]. The geomagnetic field gives the Earth partial shielding from cosmic rays, particularly from the lower energy, component that reaches only the upper atmosphere and is responsible for most of the radiocarbon production. If the geomagnetic intensity was lower than usual, then the production rate would have been higher and this would cause relevant radiocarbon dates to be too recent. In this comparison of TL and radiocarbon ages we have not, however, found evidence for a disturbance of the radiocarbon time scale caused by geomagnetic polarity excursions.

The samples were baked clay–sand ovenstones from fireplaces F6, F7, F8, F9, F12 and F14 (see refs 1 and 2 for description and location of these hearths and ovens). Initially we attempted to use the fine-grain dating technique[8]. Apparent ages in the range 30,000–50,000 yr were obtained but these were considered to be a substantial overestimate due to the presence of ('spurious') TL (not induced by radiation) in the natural glow curve; this was presumably a result of the deposited carbonate that was evident in the samples. Attempts to eliminate the spurious TL by washing in acid were not successful.

The results reported here were obtained using quartz grains in the size range 90–125 μm, extracted from the baked clay–sand matrix by magnetic separation and sieving. The procedures followed were those developed by Fleming[9] for dating quartz inclusions from pottery of the last two or three millennia, appropriately modified on account of the much greater age of the Lake Mungo fireplaces as outlined below and as will be discussed more fully elsewhere. After extraction the grains were etched in hydrofluoric acid in order to remove the outer layer of material that had been irradiated with alpha particles from uranium and thorium in the matrix. The natural TL, which was measured immediately after etching, is therefore that induced by the dose from beta, gamma and cosmic radiation received since the fireplaces were last used. The beta and gamma dose rates were evaluated from radioactive and chemical analyses of the samples and the surrounding material; a value of 15 mrad yr^{-1} was used for the cosmic dose rate. The total dose received since last heating (the archaeological dose) was evaluated by comparison of the level of natural TL in the 375 °C region of the glow curve with the (natural+artificial) TL from a second portion, the artificial TL being that induced by irradiation with a ^{90}Sr–^{90}Y beta source Studies of the TL growth characteristics of these samples indicated that it was not valid to make the assumption of linearity of TL against dose and an exponential fit to the data points was made instead of the usual linear fit; the exponential fit was of the form $(1-\exp(-x/B))$, where x is the dose and B is a constant for which the experimentally determined average value for these samples was 40 krad

Use of an exponential fit (rather than a linear one) gives archaeological doses, and hence ages, that are lower by about 18%. The primary reason for rejecting the use of a linear fit was the observation that (G_2-G_N) was on average 6% less than $2(G_1-G_N)$, where G_N is the natural TL, and G_1 and G_2 are levels of TL from portions of sample to which beta doses had been administered before any heating of the sample, and the beta doses being 4.8 and 9.6 krad respectively. The inadequacy of the linear fit was further indicated by direct observation of TL growth with

Table 1 Thermoluminescence and radiocarbon dates of ovenstones from prehistoric fireplaces

Sample	Archaeological dose (krad)	Dose rate (rad yr^{-1})	Predicted errors ($yr \times 10^{-3}$) Random	Systematic	Combined	TL date ($yr \times 10^{-3}$)	^{14}C date ($yr \times 10^{-3}$ (5,568-yr half life))
First excursion							
F7B	7.05	0.20	3.6	4.2	5.6	35.3	
F7D	6.05	0.20	2.5	3.2	4.1	29.5	30.8 ± 0.5
F7P	6.50	0.21	4.4	3.7	5.6	31.3	—
F8B	7.95	0.21	2.7	5.7	6.4	37.9	28.3 ± 0.4
F9D	7.00	0.22	4.1	3.8	5.7	32.0	—
F9J	7.60	0.23	4.1	4.2	5.8	32.3	27.5 ± 0.3
F12E	6.25	0.16	6.2	4.4	7.7	38.6	28.0 ± 0.4
			Weighted mean (TL)	=	33.5×10^3 yr		
			Standard error	=	1.3×10^3 yr		
			Predicted error (random and systematic)	=	4.3×10^3 yr		
			Average (^{14}C)	=	30.4×10^3 yr (5,730-yr half life)		
Second excursion							
F6	5.50	0.25	2.8	2.4	3.7	21.8	26.3 ± 0.5
F14P	6.50	0.21	1.7	4.1	4.4	31.4	None

The predicted error is at the 68% level of confidence and calculated as explained in ref. 10. The TL ages were evaluated on the basis that the form of the TL growth characteristic was the same for all samples.

dose using portions from which the natural TL had been drained by heating. These 'second-glow' growth characteristics were exponential in form, having a value of B of about 10 krad. This is substantially less than the value of 40 krad that corresponds to an exponential interpretation of the 6% difference between (G_2-G_N) and $2(G_1-G_N)$ mentioned above, but the higher value for B is preferred on the grounds that a change in growth characteristic often occurs when a sample is heated, particularly during the drainage of the natural TL.

The 6% difference between (G_2-G_N) and $2(G_1-G_N)$ is interpreted in terms of an exponential rather than in terms of a binomial because the former corresponds theoretically to the trapping model in which electron concentration in the conduction band is proportional to the dose rate and the TL traps are limited in number; this is the next simplest model to the one that corresponds to linear growth. It is also to be noted that the binomial function predicts a maximum in the growth characteristic rather than saturation and, to the extent that the second-glow growth characteristic can be used as evidence, this is in strong contradiction with observation.

The data are given in Table 1. The measurement precision for these samples was rather poor and the scatter in the ages is not much greater than the scatter to be expected if the samples were all of the same age. Because of this an average TL age has been evaluated for the seven samples which are considered to be associated with the first polarity excursion on the basis of radiocarbon dating (that is, F7, F8, F9 and F12). Using our standard form of citation for TL dates the date obtained for the first excursion is 35,000 b.p. (±1,300, ±4,300, OxTL 174a). The two error limits quoted, which are calculated on the usual basis[10,11], are respectively the standard error of the mean derived from the observed scatter of the individual ages, and the error predicted by taking into account all sources of uncertainty both random and systematic; the dominant contribution to the latter arises from uncertainty in the value of B.

This date is substantially more recent than the preliminary value quoted by Fleming[12] and it is not significantly different from the average of the radiocarbon dates that are available for these four fireplaces, having in mind that it is the second error limit that is relevant to this comparison, nor from the TL date of 30,400 b.p. quoted by Mortlock[13] for fireplace F7.

As regards the second excursion it should be noted that the TL date obtained for fireplace F6 is substantially different from the average date obtained for the first excursion (having in mind that between TL dates for similar contexts it is the random error limit that is relevant) but that it differs by less than two standard deviations (of the overall TL error) from the two radiocarbon dates for that fireplace. Fireplace F14, undated by radiocarbon, was assumed to be associated with the second excursion on the basis of its low intensity value; however the TL result does not support this interpretation.

Although the present work gives no evidence that these geomagnetic polarity excursions gave rise to distortion of the radiocarbon time scale the data do not exclude a major perturbation; for example, the difference of 3,100 years between the average TL age and the average radiocarbon age for the first excursion, athough within the TL error limit, could correspond to an atmospheric radiocarbon concentration 45% in excess of the level for AD 1850. However, the high geomagnetic field intensity associated with the first excursion, if global, would cause the atmospheric radiocarbon concentration to be in deficit, whereas it is only with the second excursion when the field was low that an excess would be expected.

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Evolution of Lake Abhé (Ethiopia and TFAI), from 70,000 b.p.

We summarise here the first continuous stratigraphic sequence for the late Quaternary of the African continent, derived from a detailed study of the evolution of an intertropical lake: Lake Abhé (11°N, 42°E) (Fig. 1). For the interval 20,000–0 b.p., this record complements and amplifies existing knowledge, and it also throws valuable new light on the preceding period. It provides evidence for important late Pleistocene climatic changes at the eastern end of the Sahel zone.

The results come from a multidisciplinary 5-yr project, and supporting detail is available elsewhere[1]. Analyses of diatom associations, which are sensitive indicators of ecological conditions, were carried out on 492 samples, and supplemented by convergent evidence from sedimentary facies and mineralogy. Seventy-five ^{14}C dates, derived from a 50-m core taken at the edge of the present lake (Fig. 1) and from stratigraphic sequences on the margin of the basin, provide a chronological framework with a high degree of reliability (Table 1).

Lake Abhé occupies a closed tectonic basin in the Afar (Fig. 1). The present lake ($350\ \mathrm{km}^2$) has a surface elevation of 240 m, and is currently shrinking. Only a few metres deep, it is hyperalkaline and rich in sodium bicarbonate[2]. Since it is situated at the downstream end of the perennial Awash River, which drops from a height of 2,500 m on the Ethiopian Plateau, the lake records climatic fluctuations in the Ethiopian highlands as well as in the Afar itself.

The main stages in the late Quaternary history of Lake Abhé are summarised in Fig. 2 and Table 1. Its Holocene record agrees in detail with findings from other parts of intertropical Africa[3,4]. Three (possibly four) lacustrine phases are evident. The early Holocene phase, Abhé IV, is the most important. A comparable transgression has been documented for several East African lakes[1,5-9] and along the entire southern margin of the Sahara[10-12] (Fig. 3). The Abhé IV sedimentary facies (Table 1) reflect very low turbidities, indicating that precipitation was abundant and evenly distributed through the year, as was the case throughout the Sahel zone[11,13,14]. In spite of this parallelism in rainfall, early Holocene water temperatures deduced from the diatom floras seem to have been higher in Lake Abhé and in the rest of East Africa[1,8,15] than in Chad and Mauritania (ref. 11 and B. Marciniak, personal communication). This may arise from the greater cooling of the Atlantic than the Indian Ocean during the late Wurm[16]. The brief but well defined regression ~8,000 b.p. in Lake Abhé can be connected with a fall in the level of Lakes Rudolf[5], Nakuru[5] and Chad[11] (Fig. 3) and with a change in the flow regime of the Blue Nile around 8,000–7,000 b.p. (ref. 10). It is followed by another transgression, Abhé V (Fig. 2) during which ecological conditions resembled Abhé IV although the deposits are more silty or clayey. The lake level began to fluctuate after 6,000 b.p. and fell rapidly from ~4,000 b.p. until the lake dried out. During late Holocene time, there were several minor fluctuations (Abhé VI, Abhé VII) but clastic deposits dominated. Productivity remained very low and has tended to zero during recent centuries. It is difficult to establish close correlations between Abhé VI or Abhé VII and the several minor humid phases apparent from other basins in intertropical Africa[1,3,5,11,17].

Data from Lake Abhé also confirm the existence of the hyperarid phase, synchronous with the European late Wurm, which affected intertropical Africa[1,3,6,11,12,18-23] and probably the whole intertropical zone[24-26] between 17,000 and 12,000 b.p. In the Abhé core, this arid episode is registered by a thick soil, while large debris fans formed on the basin flanks[27]. Between 17,000 and 10,000 b.p. Lake Abhé was dry.

The most important results of the Lake Abhé study relate to the period before 17,000 b.p., about which little is known in intertropical Africa. Previously, only isolated dates

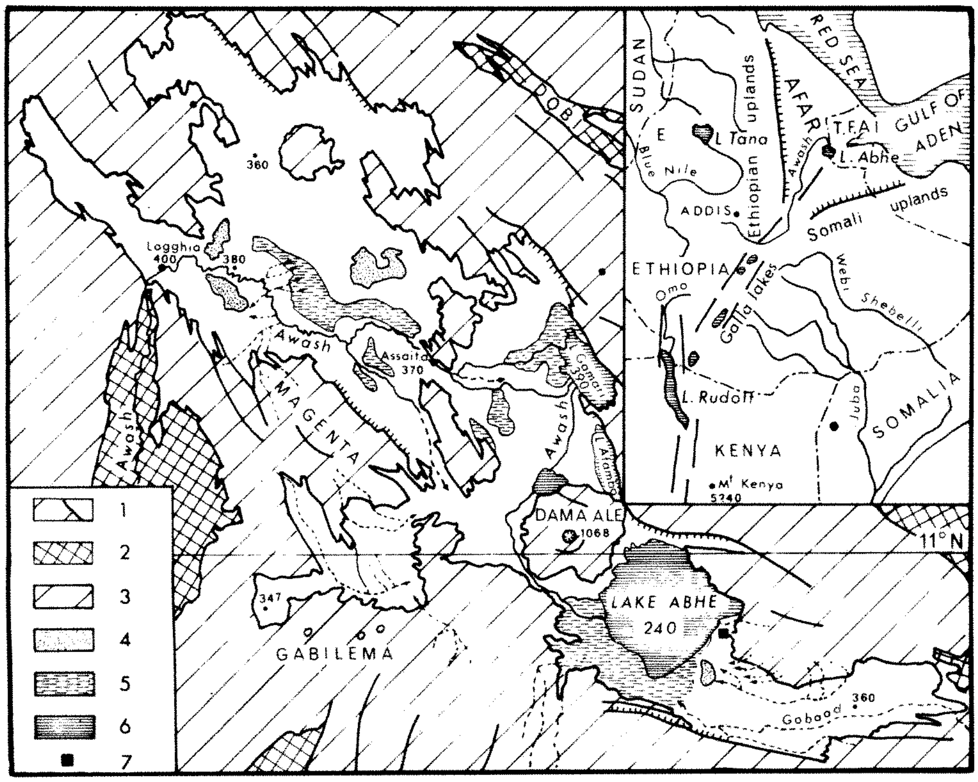

Fig. 1 The tectonic basin of Lake Abhé. 1, Edge of basin. Lake Abhé occupied almost all of this basin during the late Quaternary transgressive phases; 2, other tectonic basins; 3, basalt plateaux and volcanic massifs; 4, dunes; 5, swamps; 6, modern lake remnants 7, borehole.

Fig. 1

Table 1 The main stages in the late Quaternary evolution of Lake Abhé

^{14}C Age (yr b.p.)	Climatic episode	Sedimentary facies	Characteristic diatoms	Salt content	pH	(SiO$_2$)	Temperature	Turbidity	Productivity
★■ 110 ± 50	Modern	Clay	None	P (160 g/l)	S (10,3)	S (416 mg/l)	T (28°C)	S	11
★● 1,030 ± 100	ABHE VII	Clay CaCO$_3$(littoral)	None	M	S	S	T	S	1
✡■ 1,570 ± 60 ✡■ 2,720 ± 120	ABHE VI	Clay CaCO$_3$(littoral)	*Stephanodiscus astraea + var.*	O	M	1	T	S	M rr
		Cobble-gravel							
✪● 4,120 ± 110 ✪● 6,020 ± 160	ABHE V	Clay, Silt	*Campylodiscus clypeus, Rhopalodia giberrula*	M, P			T	S	1
● 7,190 ± 220		Clayey CaCO$_3$	*Stephanodiscus astraea var. intermedia + minutula*	O	M	1	T	M	S
✪● 7,610 ± 140		Clay	*Cyclotella striata, Melosira agassizii*	M	M	S	T	S	1
✪● 8,340 ± 180		Cobble-gravel							
✪● 8,450 ± 190	ABHE IV	Clay	None						
★● 9,380 ± 110		Pure CaCO$_3$	*Stephanodiscus astraea var. intermedia + minutula*	O	M	1	T	11	S
		Clay	*Cyclotella striata, Melosira agassizii*	M	M	S	T	S	1
✪▲ 16,400 ± 800 ✪▲ 16,100 ± 700	LATE WURM	Paleosol (Abhe core) Boulder-gravel							
✪■ 17,100 ± 400 ✪● 20,800 ± 400	ABHE III	CaCO$_3$ Calcareous silt	*Rhopalodia giberrula + var., Diploneis elliptica, Stephanodiscus astraea var intermedia*	E	M	M		S	1
⊠● 23,190 ± 170 ✪● 23,560 ± 740 ✪■ 23,900 ± 700 ✪● 24,800 ± 650 ✪■ 25,100 ± 700		Clayey calcareous diatomite	*Melosira granulata var. valida, M. nyassensis + var. victoriae, M. goetzeana*	O	M	S	T, t	S	M
✪● 26,900 ± 700		Silty diatomite	*Cyclotella ocellata, Melosira granulata*	O	M		t	S	1
✪■ 30,600 $^{+1700}_{-2200}$		Gravelly diatomite Gypsum (littoral)	*Coscinodiscus faurii*	M				S	1
✪● 31,000 $^{+1200}_{-1700}$ ✪● 34,400 $^{+2100}_{-2900}$ ✪■ > 35,000	ABHE II	Calcareous diatomite	*Cyclotella ocellata, Melosira granulata, Stephanodiscus astraea var. intermedia*	O	M	M	t	S	1
		Pure diatomite	*Nitzschia aequalis, N. stricta, N. nyassensis, N. subacicularis, Stephanodiscus hantzschii var. pusilla, Synedra rumpens var. neogena*	O	S	S	T ≥23°C	11	S
✪■ > 40,000 60,000?		Calcareous diatomite	*Nitzschia aequalis, N. stricta, Stephanodiscus astraea, S. hantzschii var. pusilla*	O	S	S		M	M
70,000?	EARLY WURM	Fluvial-sand (Abhe core) Pebble-gravel							
	ABHE I	Sandy and calcareous clay	*Stephanodiscus astraea + var*	O	M	M		S	M
		Clay Silt (littoral)	*Nitzschia eaqualis, N. stricta*	O	S	S	T	S	M
		Silty CaCO$_3$	*Stephanodiscus astraea var intermedia + minutula*	E	M	1		S	M

^{14}C-Age b.p.: only most important dates marking environmental changes are shown for the Holocene. All available dates are given for the late Pleistocene. Material dated: ●, shells; ■, well crystallised calcite or Mg-calcite; ▲, organic matter by; star in circle, G. Delibrias, Gif-sur-Yvette; ★, J. C. Fontes, Paris: open star, ref. 30: star in square, ref. 31.

Dissolved salt content: O, oligohalobe (<2 g l^{-1}); M, mesohalobe ($2<15$ g l^{-1}); P, polyhalobe (>15 g l^{-1}); E, fluctuating salinity.

*p*H: M, moderately alkaline; S, strongly alkaline.

(SiO$_2$), 1: <10 mg l^{-1}; S,: >10 mg l^{-1}.

Temperature: T, tropical; t, temperate.

Turbidity and productivity: S, strong; M, moderate; l ,low; ll, very low.

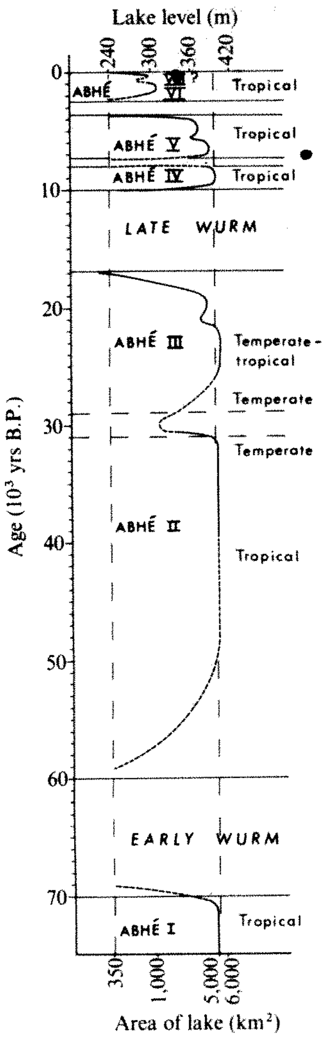

Fig. 2 Oscillations in the level of Lake Abhé from late Pleistocene to Holocene times. Based on 45 radiocarbon dates of littoral deposits for the period 0 to 35,000 b.p., and on extrapolation of mean sedimentation rates measured in the dated section of the core for the period before 35,000 b.p.

indicated high lake levels between 20,000 and 40,000 b.p. in Lake Nakuru[5], Chad[11] and Mauritania[12]. Three lacustrine phases (Abhé I, Abhé II, Abhé III), separated by drier episodes, occurred during the late Pleistocene. The earliest (Abhé I) lies beyond the range of ^{14}C dating. By extrapolating from the upper part of the Abhé core, its base (50 m) is dated at 70,000–100,000 b.p. On the other hand, the dry phase between Abhé I and Abhé II strongly resembles the arid 17,000–10,000 b.p. interval, so that it may correspond to the early European Wurm, dated by extrapolation from deep-sea cores to 60,000–70,000 b.p. (ref. 28). Abhé II began before 40,000 b.p. and was terminated by a minor regression around 31,000–30,000 b.p. (Table 1). The third phase, Abhé III, is dated between 30,000–29,000 b.p. and 17,000 b.p.

These three lacustrine phases were of similar importance (Fig. 2) but they strongly differed in their physico-chemical attributes (Table 1). The Abhé I lake was warm, alkaline, and clastic sedimentation predominates. The succeeding arid phase is marked by fluvial sands and gravels in the Abhé core and by debris fans on its margins. Abhé II was an episode of biochemical sedimentation. The lake received only material in solution, which implies gentle, well distributed rain and/or a dense vegetation cover in the catchment area. The diatom flora indicates a very warm, transparent lake with high alkalinity and productivity. Synchronous with the minor regression towards 30,000 b.p., the change in sedimentary facies was accompanied by a clear decrease in water temperatures and productivity, registered by the spread of cosmopolitan and temperate species. During the phase Abhé III, clayey calcareous diatomite accumulated in the lake. Temperate conditions persisted until 25,000 b.p. But tropical diatoms, which could support cooler temperatures than the Abhé II flora, multiplied again between 25,000 b.p. and 20,000 b.p. The ecological conditions then began to fluctuate. Numerous carbonate horizons were precipitated and brackish flora developed. The lake dried up ~17,000 b.p.

The late Quarternary evolution of Lake Abhé reflects changing precipitation–evaporation patterns. It is not yet possible to disentangle the relative effects of temperature and precipitation changes during the lacustral phases. But the episodes of greatest cold were definitely characterised by

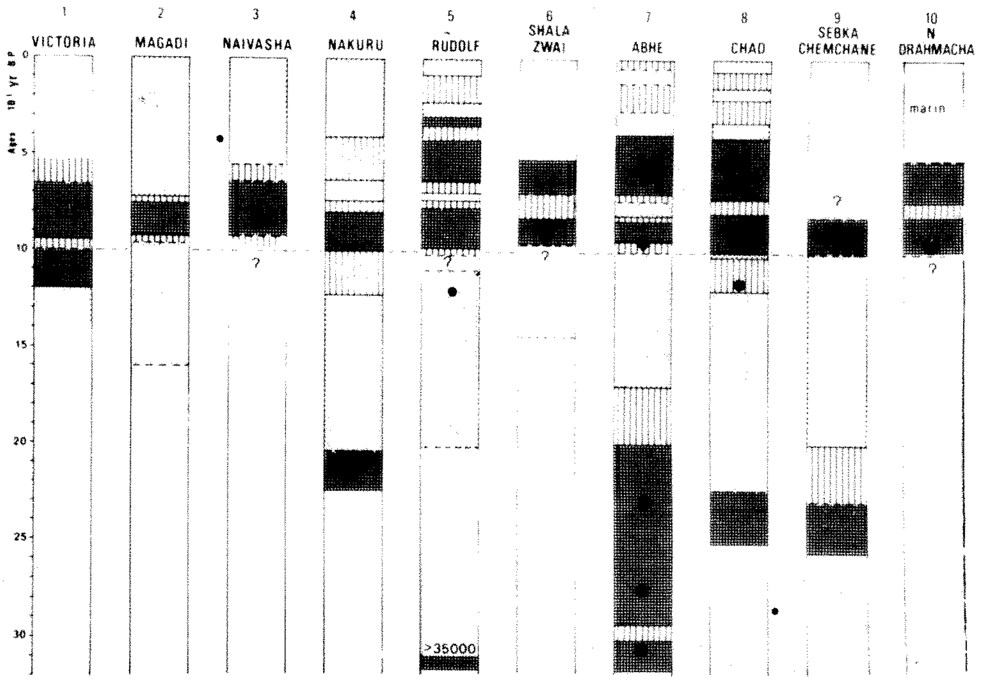

Fig. 3 Comparison between late Quaternary fluctuations of selected intertropical African lakes. From left to right the lakes grouped in order of increasing latitude (Lake Victoria 0°, Sebka Chemchane 21°N). (1) to (5) taken from ref. 5; (6) ref. 9; (7) this work; (8) ref. 11; (10) ref. 12 and B. Marciniak, personal communication. ◆, Diatom flora indicating a climate at least as warm as the present day; ●, diatom flora indicating a climate cooler than the present day.

aridity and lake-level minima. It is clear that fluctuations in the tropical atmosphere were systematically related to the high latitude glacial–deglacial sequence.

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Sulphur isotope abundance elucidate uptake of atmospheric sulphur emissions by vegetation

THE sour gas wells of Alberta form the basis of its sulphur industry. The well known Claus process is used, whereby part of the H_2S is oxidised to SO_2 which is in turn reacted with more H_2S to yield elemental sulphur. As a consequence, more than 1,200 t of sulphur per day are discharged into the atmosphere in Alberta, mostly as SO_2. Using sulphur isotope abundances, I have assessed the uptake of these emissions by vegetation.

The H_2S processed in Alberta is typically enriched in the heavier ^{34}S with δ^{34}S values ranging from + 5 to +30‰ (refs 1, 2) where δ^{34}S is defined in terms of the $^{34}S/^{32}S$ abundance ratio in meteoritic troilite as

$$\delta^{34}S(\text{‰}) = \left\{\frac{[^{34}S/^{32}S]\,\text{sample}}{[^{34}S/^{32}S]\,\text{meteorite}} - 1\right\} \times 1{,}000$$

In contrast, most Alberta soils have δ^{34}S values ranging from near 0 to −30‰ (ref. 3). Since these variations are many times the measurement precision (±0.2‰ or better), sulphur isotope abundances can be used effectively to assess the impact of the sulphur emissions on the Alberta environment.

The success of this approach is illustrated by data from the Ram River Area in Alberta where two sour gas processing plants are operational (Fig. 1). H_2S is recovered from at least two geological reservoirs by the industrial operations and the stack SO_2 has δ^{34}S values that vary from +20 to +25‰. The major peak in the δ^{34}S distribution for air in Fig. 1 thus corresponds to the industrial emissions. At a given sampling site, the atmospheric isotopic composition depends on the ratio of industrial to background sulphur oxides. The latter vary considerably in concentration and isotopic composition depending on the season, biological activity and contributions from other anthropogenic sources. The mixing ratios depend on site location and meteorological conditions. For these reasons, the histogram for air in Fig. 1 is not a sharp peak corresponding solely to the industrial emissions. The secondary peak in Fig. 1 near +11‰ will be discussed later.

Lichens (*Usnea* sp., Fig. 1) had an isotopic distribution which coincided closely with the major peak of the air. This shows that lichens take up sulphur from the air by direct pathways. One process is the interaction with the gaseous sulphur oxides. Another source is rain which has passed through sulphate formed by oxidation of the atmospheric SO_2 or dissolved particulate sulphate deposited on bark. Subsequent studies (unpublished) with *Usnea* and other genera have shown that interspecies isotopic variations are not greater than the range encountered within one species at a given site.

Upper mossy layers of the soil in this region have δ^{34}S values near +15‰, suggesting a considerable uptake of industrial emissions. Below this mossy layer (~1 cm), soil sulphur has δ^{34}S values near +2‰ indicating that moss effectively blocks the passage of sulphur compounds to the subsoil. Isotopic profiles from several soil types suggest that factors which promote penetration are lack of biological cover, net drainage and the length of time that the industrial source has been operational.

Although Fig. 1 shows that pine needles (*Pinus contorta*) had a sulphur isotope distribution of similar shape, this is shifted by

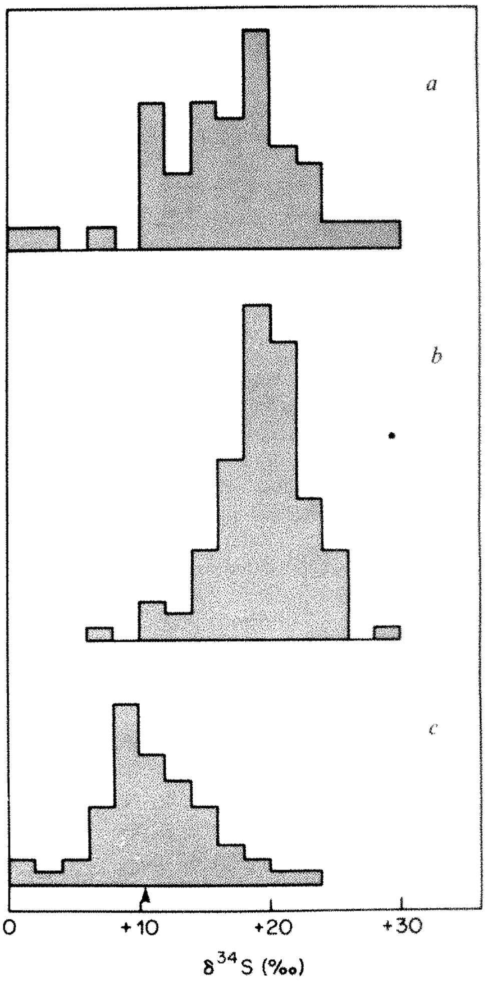

Fig. 1 Sulphur isotope variations in *a*, air; *b*, lichens and *c*, pine needles of the Ram River area for 1972–73. Atmospheric data were obtained from lead peroxide cylinders which had been set out for periods of 1 month to trap chemically the gaseous sulphur oxides. Lichens and live pine needles were gathered near the sites of the exposure cylinders. Their sulphur isotope data are based on all sulphur remaining after a brief ultrasonic washing with distilled water.

some 10‰ to lighter $\delta^{34}S$ values compared with those of air and lichens. In contrast to lichens and mosses, whose rhizines and rhizoids serve mainly for attachment, trees have root systems which transport sulphate upwards. Pine needles derive sulphur from both soil ($\delta^{34}S \sim +2$‰) and air (heavier $\delta^{34}S$ values) and therefore intermediate $\delta^{34}S$ values arise. Thus it might be argued that the data are not unexpected. But the isotopic technique facilitates assessment of the proportions of sulphur derived from the two sources. More recent data from a control site in the Ram River area, where the effects of the emissions were minimal, gave $\delta^{34}S$ values for needles and leaves similar to those of the soil ($\sim +2$‰). In another situation, sulphur compounds which originated with the industry had penetrated the soil to at least 40 cm and the conifer needles had $\delta^{34}S$ values near those of the ambient SO_2. At a given location under the influence of sulphur emissions, pine needles tend to have higher $\delta^{34}S$ values than spruce, indicating that the former derive proportionately more sulphur from the atmosphere. In the case of a very high *Pinus contorta* (~24 m) close to a sour gas processing plant (near Whitecourt, Alberta) an increase in $\delta^{34}S$ with height has been found, indicating that the canopy shields the undergrowth from the emissions.

Plots of $\delta^{34}S$ for lichens and needles compared with $\delta^{34}S$ values for atmospheric SO_2 are not as valuable for drawing conclusions as the histograms of Fig. 1. The chief reason is that the atmospheric data represent monthly averages whereas the times associated with the uptake of sulphur by lichens and needles are unknown. When certain growths can be correlated to a given period, there is no assurance that the rate of sulphur intake was uniform throughout that time. For these reasons my study lasted 2 yr in the Ram River area and needles and lichens were picked randomly near the air-monitoring stations. One concern is the extent to which the exposure cylinders are selective in the isotopes they take up especially because all field conditions are not readily simulated in the laboratory. Data obtained with high volume atmosphere sampling techniques under the auspices of the University of Calgary Interdisciplinary Sulphur Research Group (UNISUL) have been generally consistent with those obtained with lead peroxide cylinders (unpublished results). Although some of the data in Fig. 1 might have been shifted in extreme field conditions, lack of confidence in the more frequent values seems unjustified. In that the most frequent $\delta^{34}S$ values for the exposure cylinders and the lichens coincide, it would be necessary to postulate similar isotopic selectivities for both uptake mechanisms. This seems unlikely.

Several questions remain concerning the general mobility of sulphur compounds in vegetation. Pine needles can release sulphur compounds to the air and this may account for the secondary peak at +11‰ for the atmospheric data of Fig. 1, which almost coincide with the peak in the needle data. Another problem is the extent to which isotopic selectivity is involved in the uptake and release of sulphur compounds by trees. These problems are being investigated. This study was supported by the Aquitaine Company, the Atmospheric Environment Service of Environment Canada and the Canadian Forestry Service.

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Polygamy and genetic equilibrium

POLYGAMY is the normal practice in indigenous African and Islamic populations. Recently Konotey-Ahulu[1] has claimed that polygamy may explain the persistence of some abnormal haemoglobins in these populations. The generally accepted view is that the maintenance of balanced polymorphism in these populations at the loci of the commonest hereditary erythrocyte defects is due to a lesser susceptibility of the heterozygote[2-7]. An examination of that claim using a mathematical model shows that polygamy can lead to an equilibrium distribution in gene frequencies only if the male heterozygote tends to acquire more wives than either homozygote—a condition that is shown not to be unlikely in African and Islamic populations.

We consider a pair of alleles A_1 and A_2 and suppose that the associated genotypes A_1A_1, A_1A_2 and A_2A_2 have frequencies Q_{11}, Q_{12} and Q_{22}, respectively, in the present population before selection ($Q_{11}+Q_{12}+Q_{22}=1$). We assume the population is so large that the effect of random fluctuation of gene frequencies may be neglected. The generations are non-overlapping. Suppose s_1 and s_2 are the premating selection coefficients against the homozygotes A_1A_1 and A_2A_2, respectively ($0 \leqslant s_1, s_2 \leqslant 1$), then the premating genotype frequencies (after selection) are

$$q_{11}=\frac{(1-s_1)Q_{11}}{W},\ q_{12}=\frac{Q_{12}}{W},\ q_{22}=\frac{(1-s_2)_2Q_2}{W}$$

for A_1A_1, A_1A_2 and A_2A_2, respectively, where W is a normalising constant chosen to make $q_{11}+q_{12}+q_{22}=1$.

To incorporate polygamy we assume further that a man with genotype A_iA_j may marry n wives simultaneously or one after the other at random with probability

$$R_{ij}{}^{(n)}\left(\sum_n R_{ij}{}^{(n)}=1\right).$$

Let ${}_{ij}P_{rs}{}^{(n)}$ denote the conditional probability that an A_iA_j man, who marries n wives, will have an A_rA_s child. ${}_{ij}P_{rs}{}^{(1)}$ shall be shortened to ${}_{ij}P_{rs}$ for convenience. Then it follows from the condition that wives are chosen at random that

$${}_{ij}P_{rs}{}^{(n)}=1-(1-{}_{ij}P_{rs})^n \tag{1}$$

$$Pr\ (A_iA_j \text{ man marries } n \text{ wives})=q_{ij}R_{ij}{}^{(n)} \tag{2}$$

The zygotic frequencies (before selection) in the next generation are then found to be

$$\begin{aligned}
Q'_{11} &= \frac{1}{T}\sum_{n=1}^{\infty}\Big[q_{11}R_{11}{}^{(n)}\,{}_{11}P_{11}{}^{(n)}+q_{12}R_{12}{}^{(n)}\,{}_{12}P_{11}{}^{(n)}+ \\
&\qquad\qquad + q_{22}R_{22}{}^{(n)}\,{}_{22}P_{11}{}^{(n)}\Big] \\
Q'_{12} &= \frac{1}{T}\sum_{n=1}^{\infty}\Big[q_{11}R_{11}{}^{(n)}\,{}_{11}P_{12}{}^{(n)}+q_{12}R_{12}{}^{(n)}\,{}_{12}P_{12}{}^{(n)}+ \\
&\qquad\qquad + q_{22}R_{22}{}^{(n)}\,{}_{22}P_{12}{}^{(n)}\Big] \\
Q'_{22} &= \frac{1}{T}\sum_{n=1}^{\infty}\Big[q_{11}R_{11}{}^{(n)}\,{}_{11}P_{22}{}^{(n)}+q_{12}R_{12}{}^{(n)}\,{}_{12}P_{22}{}^{(n)}+ \\
&\qquad\qquad + q_{22}R_{22}{}^{(n)}\,{}_{22}P_{22}{}^{(n)}\Big]
\end{aligned} \tag{3}$$

where T is a normalising constant chosen to make $Q'_{11}+Q'_{12}+Q'_{22}=1$. ${}_{ij}P_{rs}$ can be expressed in terms of these mating

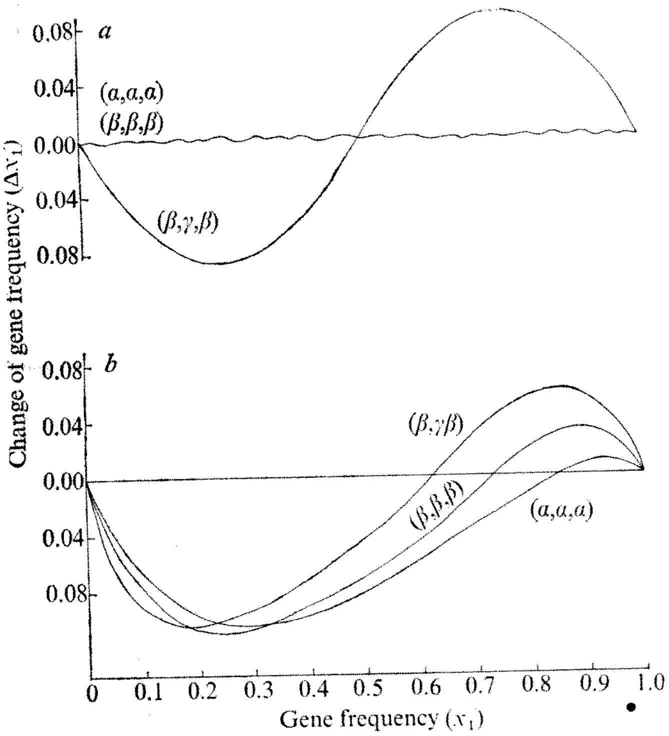

Fig. 1 Change in frequency of A_1, ΔX_1, at different values of the initial gene frequency X_1. *a*, No premating selection and no inbreeding, that is $s_1 = s_2 = F = 0$. *b*, $s_1 = 0.2, s_2 = 0.6, F = 0.1$. A graph marked (β, γ, β) refers to the situation where the conditional probability distribution of the number of wives are β, γ and β for A_1A_1, A_1A_2 and A_2A_2 men, respectively. ∝, β and γ are the hypothetical probability distributions below.

	Number of wives (n)	1	2	3	4	5	6	>6	Total
Probability distributions	∝	1	0	0	0	0	0	0	1
	β	0.5	0.3	0.1	0.06	0.02	0.01	0.01	1
	γ	0.1	0.13	0.17	0.40	0.18	0.01	0.01	1

∝: A_iA_j is monogamous; β: Half A_iA_j men marry one wife, the others marry more than one wife with decreasing probability; γ: Most A_iA_j men marry more than one wife, four being the commonest number of wives.

Note: (β, γ, β) describes the gene frequency changes for the situation where A_1A_2 has a greater probability than A_1A_1 and A_2A_2 to acquire many wives.

frequencies as shown in Table 1. So we can write Q'_{ij} explicitly in terms of the Q_{ij} values. The set of equations (3) then specifies the relationship between the genotype frequencies of any generation and that of the preceding generation. The form of equation (3) indicates that polygamy will not affect the behaviour of the system unless $R_{11}^{(n)}$, $R_{12}^{(n)}$ and $R_{22}^{(n)}$ differ. General solutions to the system could not be found. Some of the results of numerical calculations are illustrated in Fig. 1.

We note (Fig. 1*a*) that when there is no premating selection and no inbreeding, and every man marries one wife, every point is at equilibrium. This is consistent with the Hardy–Weinberg equilibrium law that a large population under random mating reaches an equilibrium no matter what the initial allelic frequencies may be. Other cases showed that polygamy will not produce a stable equilibrium in the absence of premating selective forces, except for the cases where the heterozygotes tend to acquire more wives than the homozygotes.

Our model is obviously oversimplified, but it does show that where the male heterozygote has a greater tendency to acquire more wives than either homozygote we can have non-trivial equilibrium at intermediate gene frequencies. This is in fact another form of differential selection in which heterozygotes are favoured over the homozygotes, and supports the hypothesis put forward by Fisher[8] to explain the phenomenon of balanced polymorphism. In effect, then, our model is equivalent to one in which selection operates differently in males and females as is the case with Owen's and Mandel's generalisation of it[9-11]. However, ours has the advantage of incorporating polygamy directly, and considers selection really in two stages: premating selection, and selection in the number of mates. This is important in the discussion on the maintenance of genes for abnormal haemoglobins in populations where malaria is endemic and polygamy is practised. Differential polygamy in which male heterozygotes show a greater fitness by virtue of their potentiality to acquire more wives, will enhance the effect of premating selective forces which favour the heterozygotes, in the maintenance of genetic variability in the population.

Table 1 Calculation of ${}_{ij}P_{rs}$

Man	Offspring	Wife A_1A_1 (q_{11})	Wife A_1A_2 (q_{12})	Wife A_2A_2 (q_{22})	${}_{ij}P_{rs}$
A_1A_1	A_1A_1	1	$\frac{1}{2}$	0	$q_{11} + \frac{1}{2}q_{12}$
	A_1A_2	0	$\frac{1}{2}$	1	$\frac{1}{2}q_{12} + q_{22}$
	A_2A_2	0	0	0	0
A_1A_2	A_1A_1	$\frac{1}{2}$	$\frac{1}{4}$	0	$\frac{1}{2}q_{11} + \frac{1}{4}q_{12}$
	A_1A_2	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}q_{11} + \frac{1}{2}q_{12} + \frac{1}{2}q_{22}$
	A_2A_2	0	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}q_{12} + \frac{1}{2}q_{22}$
A_2A_2	A_1A_1	0	0	0	0
	A_1A_2	1	$\frac{1}{2}$	0	$q_{11} + \frac{1}{2}q_{12}$
	A_2A_2	0	$\frac{1}{2}$	1	$\frac{1}{2}q_{12} + q_{22}$

In African and Islamic populations malaria is prevalent. The heterozygotes are therefore more likely to survive childhood than the normal homozygotes. These heterozygotes will tend to acquire more wives than the normal homozygotes because about half the children a heterozygous man has with a homozygous recessive woman will be homozygous for the unfavourable allele, and about one-quarter of the children he has with a woman who is heterozygous will be homozygous for the unfavourable allele. Most of the children homozygous for the unfavourable allele used to die very early and, since in African populations procreation is the *raison d'être* of marriage, the heterozygote would tend to marry more wives, in the hope that the children he gets with his new wives will 'stay'. Our analysis supports the view of Konotey-Ahulu[1] that polygamy could be one of the factors in the maintenance of the sickle gene in African and Islamic populations. It will enhance the effect of premating selective forces.

In our analysis we assumed hypothetical values for the probability that an A_iA_j man would marry n wives. The Ghana Institute of Clinical Genetics, Accra, is collecting data that would enable us to estimate these probabilities in African populations.

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Lek behaviour in three species of the subgenus *Hirtodrosophila* of Australian *Drosophila*

LEK behaviour, or the use of courting territories, is well known in the patterned wing, Hawaiian species of the subgenus *Drosophila*, but not elsewhere in the genus[1]. Australian *Drosophila* comprise a major adaptive radiation in the subgenus *Scaptodrosophila*, and minor radiations in *Hirtodrosophila* and *Sophophora*[2,3]. We report here on three species of the subgenus *Hirtodrosophila*, two with patterned wings and one without, that show lek behaviour.

The species are found in rain forest habitats from eastern Victoria to northern Queensland, a latitude range of 37°S–16°S; floristic composition of the rain forests varies widely over this range[4]. In especially damp and shaded regions, however, bracket fungi (Polyporaceae) of various species occur, growing laterally from the sides of fallen logs or tree trunks close to the ground[5]. The three *Hirtodrosophila* species use the horizontal undersides of the fungi as leks. Males position themselves on the undersides of fungi at regular intervals; females from the surrounding vegetation occasionally land there, and courtship activities follow. The undersides of the fungi are white or light grey, strongly enhancing displays; flies have not been found on old fungi with dark undersides.

During courtship, wing display is prominent. Two species, *D. mycetophaga* and *D. polypori*, have patterned wings, and both are commonly found from eastern Victoria to southern Queensland (latitude 2°S)[3]. The third species, *D. mixtura*, however, occurs only in northern Queensland. Its wings are tinged brownish especially towards the costal margin, but in contrast to other Australian *Hirtodrosophila* species and the Hawaiian species, it is striking in appearance, being very dark above, and changing abruptly to unicolorous pale cream below. Like the patterned-wing species, therefore, flies stand out strikingly when displaying on the undersides of bracket fungi.

In addition, two other as yet undescribed and rarer species have been collected from under bracket fungi, one with patterned wings, and one very closely related to *D. mixtura*. Presumably, they too use the fungi as leks. It is remarkable that only three patterned-wing Australian *Hirtodrosophila* species are known, all apparently using the same defined courting territories.

The five species are somewhat larger than many other Australian endemic species[3]. In contrast to the Hawaiian patterned-wing species[1], sexual dimorphism has not evolved, except that males are somewhat smaller than females. A striking level of sexual dimorphism has presumably evolved in the Hawaiian species, because the advertising males occupy sites in foliage to attract females to these seemingly less well defined courting territories.

We conclude that two patterned-wing species in the southern part of Australia, and a non patterned-wing species in the north show lek behaviour, the leks being identical in type. Moreover Hawaiian lek species belong to the subgenus *Drosophila* suggesting that convergent evolution has occurred in two subgenera of the genus for a behavioural pattern of considerable evolutionary significance which is unreported elsewhere in the genus.

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Longevity and lifetime body weight in mice selected for rapid growth

AN association between lifespan and early growth rate as affected by nutritional manipulation has been reported; high intake–rapid growth apparently contribute to shorter lifespan, and low intake–slow growth to longer lifespan[1,2]. Genetically determined rapid growth has also been reported to be associated with shortened lifespan[3].

A strain of mice with approximately double normal growth rate, and with modified food intake, body composition and reproduction patterns has been developed by selection for body weight gain from 21–42 d of age[4]. We report here data on the lifespan and lifetime body weight of this strain (G) developed by us, and of contemporary animals from an unselected random bred strain (C) from the same base population.

The study involved 30 litters of line C and 38 litters of line G, from generation 33 of both lines. Two mice of each sex were sampled from each litter, and weighed weekly beginning the day after birth, to 16 weeks. From weaning, at three weeks, they were housed four to a cage, by sex and strain. At 16 weeks, numbers were reduced by random sampling within litters to a total of 16 per sex per strain, representing 30 and 32 litters for C and G respectively, and the number per cage was reduced to two. 'White diet', with a guaranteed analysis of not less than 24% crude protein and 6% fat, and not more than 3.5% crude fibre, was fed *ad libitum* throughout the experiment.

The 64 animals selected at 16 weeks were weighed at 4-week intervals until they died from 'natural' causes. An autopsy was carried out on the majority of the animals, and the incidence and location of tumours and other abnormalities noted.

Mean life span for both lines, by sex, is given in Table 1. On average, animals from line G had a life span only 57% that of those from line C.

Table 1 Mean lifespan (weeks) for two strains of mice: control (C) and rapid 3–6-week body weight gain (G)

Line	Males		Females	
	n	$X \pm$ s.e.	*n*	$X \pm$ s.e.
C	16	112.1 ± 5.4	16	119.5 ± 3.8
G	16	51.1 ± 5.7	16	82.3 ± 3.2

Since all animals of both strains in this study had the same diet, the results are in agreement with the finding[2] that growth rate is more important than dietary composition in influence on lifespan.

There was a significant interaction between line and sex in that females in line G lived about 60% longer than males, while the two sexes in line C did not differ significantly in longevity.

Previous work with these strains had shown that line G mice have significantly higher food intake and body fat percentage than line C[5–8]. Based on data from nutritional manipulation experiments with rats^{1,2}, it seems probable that the premature ageing in line G is associated with obesity.

A variety of tumours, in terms of tissues and sites, was observed at autopsy; a pattern already noted in rats^{2}. The numbers of animals found to have tumours when autopsied was similar in the two sexes, and non-significantly higher in line G (48%) than in line C (39%). Since the life span in line G was considerably shorter than that in line C, it seems that tumours develop earlier in line G, and probably more frequently. A relation between tumour risk and calorie intake in rats is known to exist^{9}.

Mean lifetime body weights for both lines and both sexes are shown in Fig. 1. The number of animals for determination of the means decreased with time, and thus the reliability of the estimates of line mean weights becomes progressively less. However, lifetime growth curves for the later surviving mice were very similar to those shown for all mice, and the difference between the two strains in growth pattern is clear. Selection for rapid 3–6-week gain resulted in a considerably higher mature weight in line G than in line C. Line G mice attained maximum weight at an earlier age than those in line C, and then promptly declined, to a value about 40% below the maximum and near that of the control. In both lines, males attained maximum weights at earlier ages than did females.

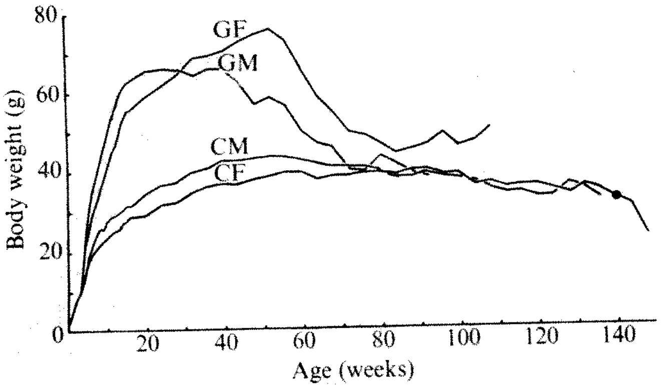

Fig. 1 Mean body weight (g) plotted against age (weeks) for males and females of two lines of mice: control (CM and CF) and rapid growth (GM and GF).

Roberts^{3} studied longevity in mice selected for increased body weight. All animals were mated in that experiment, so the results on longevity cannot be compared with those in this study, at least with regard to females, and possibly also not for males^{10}. Roberts' results are, however, very similar to ours with regard to the earlier attainment of maximum body weight in animals selected for rapid growth, followed by a marked decline in weight beginning shortly after attainment of maximum weight and continuing until death. This decline he attributed to a depletion of fat reserves, also indicated by the appearance of our mice.

Experimental studies of longevity are of necessity costly, and time consuming. Male mice of strain G described here had a mean lifespan, under good husbandry conditions, of just under one year, which may represent the shortest lifespan available in a mammalian species readily available for experimentation. This, coupled with the fact that the strain is known to differ from the the unselected control in food intake, body composition, food conversion and certain other physiological parameters, and that the pattern of tumour incidence at death is apparently quite similar to that in rats with nutritionally induced variation in lifespan, suggests that it may be a particularly useful model for studying causal relationships between food intake, growth, obesity, and ageing.

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Naloxone-induced inhibition of ethanol dependence in mice

It has been suggested that tetrahydroisoquinoline (TIQ) alkaloids formed *in vivo* after ethanol ingestion represent metabolic sequelae linking the types of addiction caused by ethanol and the opiates^{1,2}. The theory derives from the fact that biosynthesis of opiate alkaloids involves TIQ intermediates^{3}, and that TIQ formation from β-arylalkylamines and aldehydes proceeds rapidly at physiological *p*H (ref. 4). There have been strong criticisms of this hypothesis$^{5-7}$, but the demonstration of an effect on opiate activity by certain neuroamine-derived TIQs$^{8-10}$ prompted us to investigate the question further. Specifically, it was interesting to know the effect of naloxone, an agent known to block the development of dependence to opiates^{11}, on the development of dependence on ethanol.

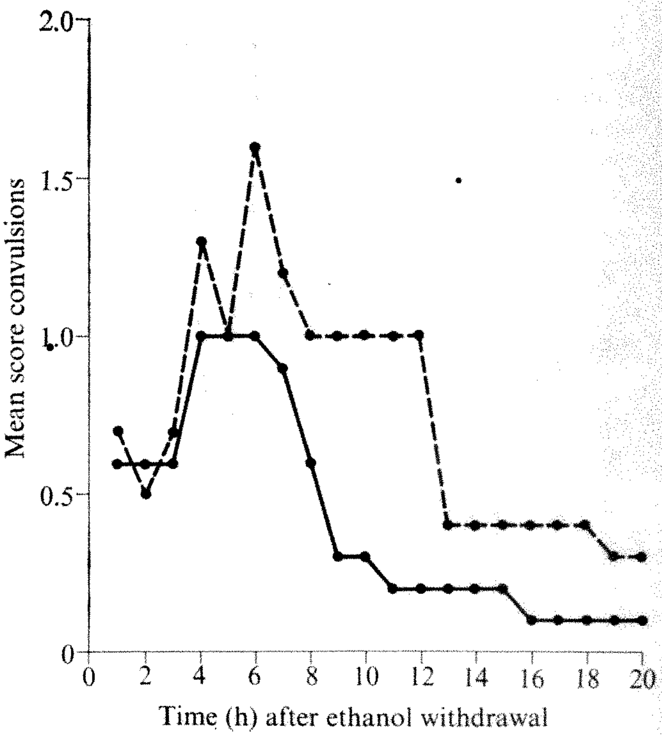

Fig. 1 Effects of chronic naloxone injection of the ethanol-induced withdrawal response in mice (——). Naloxone (2 mg kg^{-1}) was injected intraperitoneally every 6 h for 1 d before exposure to ethanol and every 6 h for 3 d during exposure for a total of 16 injections. Control mice (- - - - - - -) were injected with saline at the same time and with the same volume as the naloxone-treated mice. At least ten mice were used in each group.

Physical dependence on ethanol was induced in male Swiss-Webster mice (18–25g) by the technique of Goldstein and Pal[12]. Groups of 24 mice were housed in airtight chambers and exposed to ethanol vapour carried by a slow stream of air for 3 d. Daily throughout the experimental period, concentrations of ethanol in blood and air were determined[14]. The animals received either naloxone (2 mg kg^{-1} in saline) or equivalent volumes of saline every 6 h for 1 d before exposure to ethanol vapour, and every 6 h for 3 d during exposure. A priming dose of ethanol (1.67 g kg^{-1}, 25% v/v) and pyrazole (68 mg kg^{-1}), a compound known to inhibit ethanol metabolism and thus ensure stable blood ethanol concentrations[13], was injected intraperitoneally on the first day of exposure to ethanol. Drug doses were calculated as the base form rather than the salt. On the second and third days, the mice were removed from the chamber once a day for 45 min for collection of tail blood samples and the single daily injection of pyrazole, and every 6 h for injections of naloxone or saline. Withdrawal was induced on the fourth day by removal of the mice from the ethanol chambers. Withdrawal severity, as measured by convulsions on handling has been described. Pyrazole control experiments, in which pyrazole was injected daily (68 mg kg^{-1}) without ethanol vapour, produced no significant convulsions when the drug was withdrawn on the fourth day.

The 6-h injections of naloxone significantly inhibited ($P < 0.01$) convulsions in response to withdrawal of ethanol. Scores were lower (14 times) at peak differences than those of the paired saline control. The mean convulsion score for saline was 0.76 ± 0.05, whereas that for naloxone was 0.46 ± 0.04 ($P < 0.01$), showing marked inhibition of ethanol dependence in mice (Fig. 1).

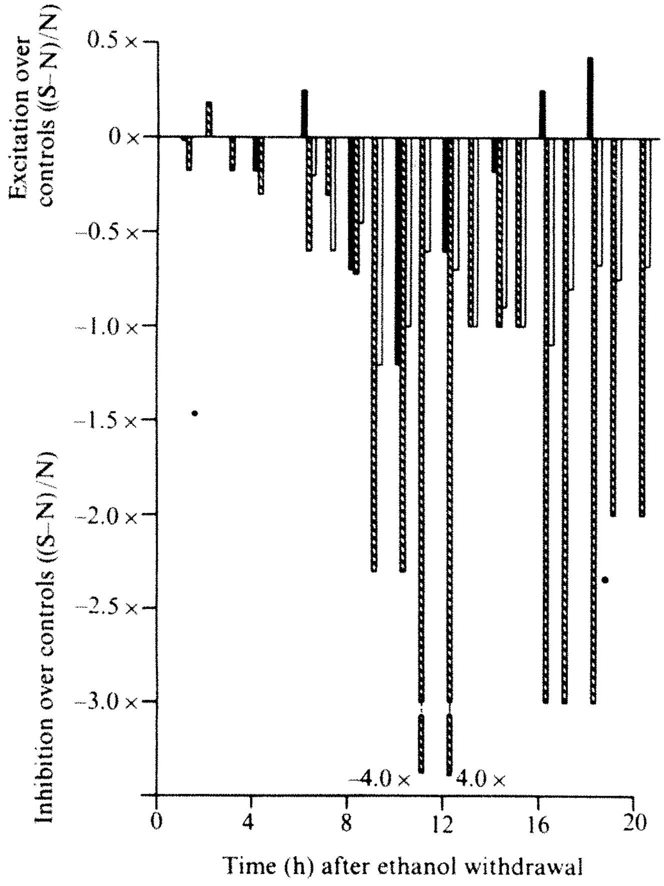

Fig. 2 Effects of naloxone on ethanol withdrawal convulsions from each of three experiments. The fraction ((S – N)/N) represents withdrawal severity and is calculated from

$$\frac{\text{mean saline convulsions} - \text{mean naloxone convulsions}}{\text{mean naloxone convulsions}}$$

for each of the 20 h listed (see text). Twenty-four mice were used for each experiment. Open columns, experiment 1; hatched columns, experiment 2; filled columns, experiment 3.

To illustrate this further, a composite of three experiments with a total of 72 mice is represented as a histogram in Fig. 2. The experimental protocol was the same as that described above. Naloxone (2 mg kg^{-1}), administered in four divided doses, reduced the withdrawal convulsions consistently and reproducibly in triplicate experiments. We calculated a ratio representing withdrawal severity (WS) according to the following formula

$$\text{WS} = \frac{\text{saline withdrawal score} - \text{naloxone withdrawal score}}{\text{naloxone withdrawal score}}$$

$$\left[\frac{(\text{S}-\text{N})}{\text{N}}\right]$$

When $\text{WS} > 0$, the withdrawal convulsions have been inhibited; when $\text{WS} < 0$, the convulsions have been exacerbated. Fig. 2 shows the percentage inhibition (or excitation) of WS by naloxone during withdrawal from ethanol.

To analyse ethanol in the blood and brain of mice undergoing withdrawal from ethanol vapour, two groups were injected with either saline (equivalent volume as naloxone) or naloxone (2 mg kg^{-1}) every 6 h for 4 d, three of which were during exposure to ethanol. These mice also received daily injections (68 mg kg^{-1}) of pyrazole during exposure, as before. Blood and brain samples were taken from these groups 5, 10, and 15 h after withdrawal. Mice were killed and blood and brain samples from each were analysed for ethanol by gas chromatography[14]. At least five mice were used for each determination.

Ten hours after withdrawal (the time of peak seizure scores) the concentrations of ethanol in the blood or brain after administration of saline were 41.5 ±3 mg dl^{-1} and 35.7 ±4 mg dl^{-1}, respectively; these values do not differ significantly from those obtained after administration of naloxone, which were 47.5 ±2 mg dl^{-1} and 43.7 ±2 mg dl^{-1}, respectively. Similarly, naloxone did not affect the concentrations of ethanol at 5 h after withdrawal. Thus, the effect of naloxone on convulsions induced by withdrawal of ethanol do not seem to be related to an alteration of ethanol metabolism.

We next analysed brain calcium in mice given naloxone or saline and then exposed to ethanol vapour using the modified wet ash procedure of Cardenas and Ross[21].

Mice were divided into two groups of twelve. Group *A* received an injection of naloxone (2 mg kg^{-1}) every 6 h, and group *B* received an equivalent volume of saline. They were treated 24 h before exposure to ethanol. The injection schedule continued daily for 3 d before withdrawal convulsions were recorded. Pyrazole (68 mg kg^{-1}) was injected as before.

Table 1 shows that chronic exposure to ethanol produces a significant decrease ($P < 0.01$) in brain calcium, to 70% of control in the first 24 h. On the second and third day of ethanol exposure, the effect was similarly significant ($P < 0.05$), though less effective, depleting calcium to 78 and 84% of control values, respectively. Naloxone, which by itself has no significant effect on brain calcium levels, prevented the ethanol-induced depletion of brain calcium ($P < 0.05$). These results suggest that the effect of naloxone on ethanol-induced withdrawal convulsions is mediated, at least in part, through an action on calcium metabolism in the central nervous system.

The finding[7] that naloxone, an agent known to precipitate jumping in morphine-tolerant mice[15], does not have this effect when administered acutely to ethanol-tolerant mice, cast doubt on the alkaloid biogenesis theory linking opiate and ethanol actions. Nonetheless, several lines of research have produced evidence that argues for such a relationship. Thus, cross-tolerance betweeen morphine and ethanol[16], increased tolerance

Table 1 Naloxone blockade of chronic ethanol-induced depletion of brain calcium in mice

Treatment	Brain calcium during chronic ethanol exposure* (mg g^{-1} (mean±s.e.m.) wet weight)		
	Day 1	Day 2	Day 3
Saline (equiv. volume to naloxone+ethanol vapour)	36.0±4.8†	39.9±0.3†	43.2±1.6†
Naloxone+ethanol vapour naloxone was administered at 2 mg kg^{-1} every 6 h (24 h before and every day during ethanol vapour exposure)	51.0±2.6‡	45.8±3.8‡	45.1±4.8‡

Ten mice were used for each determination.
Calcium value for control brain = 51.0±6.3 and for naloxone (2 mg kg^{-1}) = 52.2±2.0.
* Mice were killed 24 h apart (day 1 = 24 h exposure, day 2 = 48 h and day 3 = 72 h).
† Values significantly different from controls ($P <$ at least 0.05).
‡ Values not significantly different from controls.

to morphine in ethanol-habituated animals and man[17], inhibition of ethanol withdrawal convulsions by morphine[18], and attenuation of ethanol-induced narcosis by naloxone[19] have all been demonstrated. Furthermore, the TIQ alkaloid, 3-carboxy-salsolinol (the L-dopa-acetaldehyde adduct) potentiates ethanol-induced narcosis[20] and morphine analgesia[10] in mice, and salsolinol (the dopamine acetaldehyde adduct) interacts with the opiate receptor of the guinea pig ileum[9]. Our demonstration of inhibition of ethanol-induced withdrawal convulsions and blockade of ethanol-induced calcium depletion by naloxone provides further evidence for a functional relationship between opiates and ethanol.

We emphasise, however, that the results of these experiments and the others mentioned here, showing antagonism by naloxone of non-opiate drugs, must be viewed with caution. It is possible that naloxone, although considered to be a specific opiate receptor antagonist[11], has other actions so far unappreciated. The results would then simply reflect the action of naloxone at this alternate site(s) of action.

Nonetheless, the present state of knowledge supports our interpretation that ethanol and the opiates share some common biochemical mechanisms, and further research to delineate these mechanisms is warranted.

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Frog retinal ganglion cells show species differences in their optimal stimulus sizes

THE purpose of this study was to compare the optimum stimulus sizes of class 2 ganglion cells in *Rana pipiens* with those of *R. catesbeiana*. Several neurophysiological studies have suggested that activity in class 2 ganglion cells in the frog retina (convexity sensitive[1–3]) serves as a trigger for prey catching[1,4]. Field studies of the stomach contents of various species of frogs show that the prey selected by the bullfrog, *R. catesbeiana*, differs from that of the leopard frog, *R. pipiens*, in size. Bullfrog stomachs have contained (in addition to insects) crayfish, frogs, mice, and birds[5,6]. Leopard frogs generally prey on various insects, spiders, and terrestrial snails[7,8]. This study was undertaken to explore a possible difference in the electrophysiological optimal stimulus size for class 2 retinal ganglion cells of leopard frogs and bullfrogs which might be related to differences in prey size preference. Our data suggest that bullfrog ganglion cells have average optimal stimulus sizes significantly larger than those of leopard frogs.

The experiments described were conducted on adult frogs of northern North American origin. Leopard frogs ranged in body weight from 14.0 to 46.5 g, while bullfrogs weighed from 222 to 440 g. All frogs were acclimatised in the laboratory at 20–22 °C. for at least two weeks before use and were fed every two days. The animals were anaesthetised for the duration of the surgery with MS:222. The cranium was opened to expose the tectum. Paralysis was achieved with injection of neuromuscular blocking agents and xylocaine was infused into the incision site. MS:222 was then discontinued for the recording period. Frogs were dark-adapted for 1 h before recording began. Conventional electrophysiological techniques were used to record from optic nerve terminals in the tectum[9]. Platinum-plated, alloy-filled glass micropipettes of 3–5-μm tip diameter[10] were employed for the recordings. Electrical data were recorded on an A.M. tape recorder for later analysis. Spike trains from single neurons were resolved with a pulse height discriminator and the action potentials counted using a PDP-11 data acquisition computer system.

Visual stimuli consisted of black, round spots projected on to the back of a translucent tangent screen situated 24 cm from the frog's eye. Background illumination of the screen measured at this distance with a Weston Model 614 foot-candle meter was 3–4 ft cd. The spots were produced photographically on a transparent film strip and ranged from 2.3° to 28° in diameter (i.e. the angle which the diameter of the spot subtended at the eye) in seven discrete sizes. Each size was presented twice, with 35 s allowed between presentations. A motor-drive moved the filmstrip through a projector so that the spots travelled horizontally across the screen at a constant, 7° s^{-1} angular velocity, through the centre of the excitatory receptive field (ERF) of each cell.

The topography of the retino-tectal projection onto the optic tectum was found to be similar in the bullfrog and leopard frog, and the same as described for other Ranid species[11,12]. In both species, units were recorded from the mid-lateral region of the tectum which receives input from the central visual field to avoid disparities which might be caused by sampling from different fields. Class 2 cells were identified according to the following criteria: the cell responded best to small moving objects: it showed a sustained, movement-gated response to a stationary stimulus positioned in the ERF[8]; the sustained response had to be 'erasable', i.e. transient darkness would permanently extinguish the on-going response[8,9]; and the cell showed a brief response to the general 'OFF' of light, but a weak, habituating response to the 'ON'. After the cell was identified as a class 2 type, the approximate extent of the ERF was mapped with a 2° spot. Then the response of the cell to each of the seven different stimulus sizes was determined. The computer was used to count individual spikes produced by the cell and also to measure the between-spike intervals. Spikes occurring after intervals longer than 50 ms were discarded and the response was then scored as the number of action potentials greater than or equal to 20 Hz. This frequency was chosen in order to eliminate background and low level activity. The cell's response was plotted against the logarithm of the area of each stimulus spot (Fig. 1). In order to determine the optimal stimulus size from the experimental data, we fitted a quadratic function to each cell's response profile (Fig. 1), using a computerised, stepwise, polynomial regression procedure. The goodness of the fit was tested for each case by an F test. All values of F so obtained were greater than 5.7 (d.f. 2/11) which is significant at better than the 0.025 level (most values exceeded the 0.01 level). By differentiating the quadratic equation, we calculated the maximum of the curve which lies over the stimulus area that elicited the maximum response. From this area, we calculated the diameter of the optimum stimulus spot size in degrees for each cell, individually.

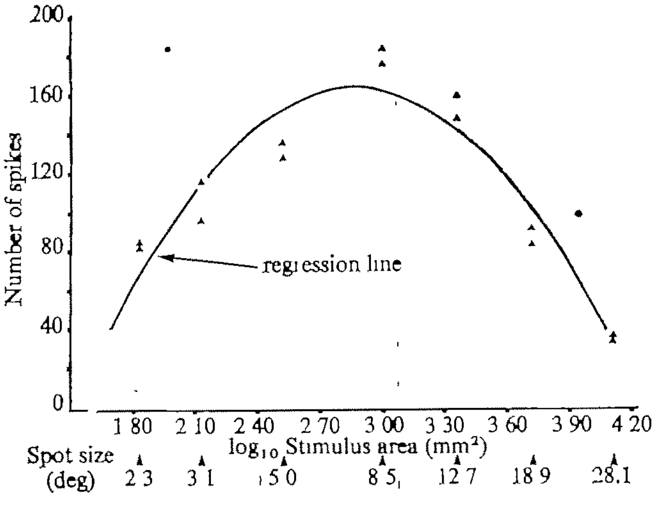

Fig. 1 Data obtained from one bullfrog class 2 ganglion cell. This figure demonstrates the technique used to determine the optimal stimulus size for the neuron. ▲, The response of the cell to the various spot sizes used. Each spot size was tested twice. The curve is the quadratic function fitted by a computerised polynomial regression procedure ($F = 46.4$) For a quadratic equation of the form $y = a+bx+cx^2$, the point of zero slope, $dy/dx = 0$, is given by $b+2cx = 0$. This yields $x = -b/2c$ which predicts the maximum of the curve. In the case illustrated above, the maximum lies over the log stimulus area of 2.88, corresponding to a spot size of 7.4° or a stimulus area of 758.6 mm^2 The equation of the regression line shown is

The results from 25 cells from 8 leopard frogs and 31 cells from 12 bullfrogs show that the optimum stimulus sizes of leopard frog class 2 cells average 5.22° (s.e. 0.3°), while the average for the bullfrogs is 7.77° (s.e. 0.21°). These values indicate that the bullfrog optimum stimulus requires a 50% larger diameter, or a 100% larger area than the optimum for leopard frogs. The null hypothesis that these two samples come from the same population may be rejected at $P < 0.001$ (Mann-Whitney U test). Our size optimum found in *R. pipiens* is comparable to the 3°–5° optimum found in *R. esculenta* class 2 cells by Butenandt and Grüsser[13]. This European frog is similar in size to the leopard frog. More interesting, perhaps, than the mean of the optimal stimulus sizes is the distribution of these sizes (Fig. 2). Although there is overlap of size optima between both species, bullfrogs exhibit many cells with larger optima. Of our sample, 74% of bullfrog cells responded best to objects larger than 7°, whereas 84% of leopard frog cells responded best to objects smaller than this size.

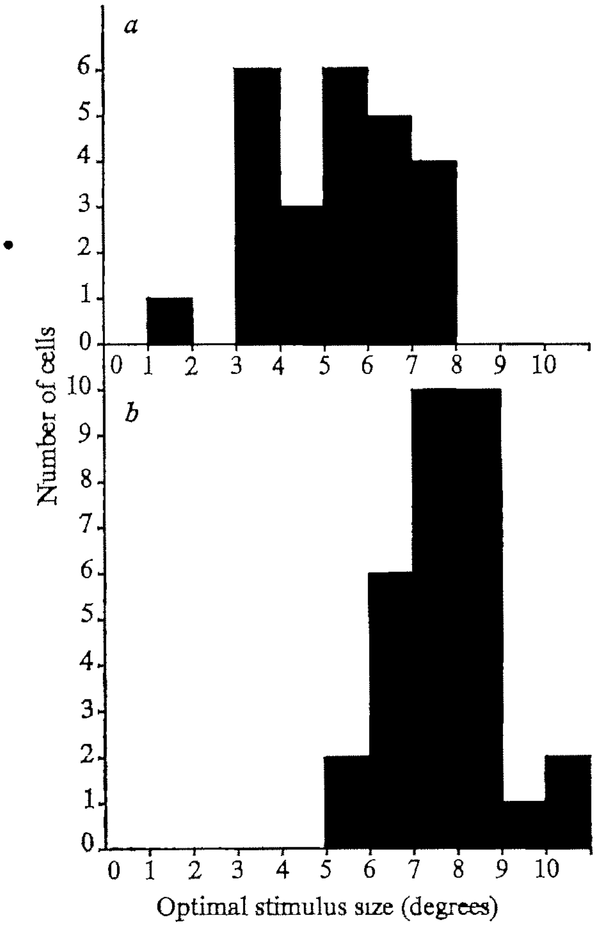

Fig. 2 Distribution of optimal stimulus sizes for 25 leopard frog (*a*) and 31 bullfrog (*b*) class 2 retinal ganglion cells determined by single unit recording of the responses of these cells to spots of different sizes moved across their receptive fields at a velocity of 7°/s. The average optimum stimulus size for the bullfrog cells was 7.77°, compared to 5.22° for leopard frog cells. Of bullfrog cells, 74% had size optima exceeding 7°, while 84% of leopard frog cells had a lower size optima.

Two conceivable difficulties with the interpretation of our results deserve mention First, we may not have sampled cells from comparable parts of the retina in both species of frog. Across many species of vertebrates, receptive field sizes of

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ganglion cells increase from retinal centre to periphery[14]. Optimum stimulus size is roughly proportional to receptive field size in the frog retina[13]. If the optic axis of the bullfrog is different from that of the leopard frog, we could have sampled cells from non-analogous retinal regions, thereby introducing a possible discrepancy into our measurements. However, the similarity of the retinotectal projection between the two species which we have observed, and the similarity of their visual fields of view as revealed by perimetry[15], lead us to doubt that a significant difference exists in the optic axis. Secondly, there is the possibility that the two species of frogs studied have had different refractive errors, resulting in varying degrees of retinal defocusing when both animals viewed the projection screen at the same 24 cm distance. We performed reflection retinoscopy on paralysed frogs and detected consistent, but small differences in the refractive errors of the two species. Leopard frogs were slightly myopic and bullfrogs were slightly hypermetropic. The errors measured however were less than 1.5 diopters and therefore were not considered to be large enough to defocus seriously the retinal image or to cause substantial disparities in retinal image size.

Technically, we note that the retinal image size of a given object at a constant viewing distance will be larger in the bullfrog eye than in the leopard frog eye, assuming that the refractive indices of cornea, lens, and vitreous are identical in both species. The linear extent of the image on the bullfrog retina will exceed that of the leopard frog in the same proportion as the ratio of the posterior focal lengths of their eyes. This disparity in size would lead one to predict that bullfrog retinal ganglion cells, if there were no differences in retinal circuitry, would have smaller optimal stimulus sizes than those of leopard frogs. Our observation, however, that in fact the reverse is the case, supports the existence of such a difference.

Behavioural experiments have indicated that, in addition to size cues, other factors are important for initiating prey catching in frogs. Olfactory input suggestive of prey can influence toads to choose larger than usual prey objects[16]. Motivation to feed may affect size preference[17] as does satiety level[18]. The contrast of the object with the background as well as the season is significant[19]. 'Predator-like' visual stimuli excite neurons in the thalamus and pretectum which in turn inhibit tectal neurons, thereby blocking prey catching[20,21]. Single unit recording of retinal ganglion cells cannot elucidate all the complexities of prey catching that behavioural experiments have revealed (see, for example, Ingle[22]). Prey catching does not follow activity in class 2 fibres in a one to one fashion. It is true, however, for the frog retina, that highly processed information about a visual stimulus is presented to the brain. We hypothesise that encoded in class 2 responses are size cues which may be one of several important triggers for prey catching. The differences in the size cues emitted by the retinae of frogs of different species may be the first step in producing a difference in prey size preference.

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Normal postimplantation development of mouse parthenogenetic embryos to the forelimb bud stage

PRE- AND POSTIMPLANTATION development of parthenogenetic embryos is of interest in analyses of the role of the male genome in early mammalian development, differentiation, and in haploid and diploid gene expression in embryogenesis. Diploid and haploid mouse parthenogenones evidently possess the capacity to form teratomas and give rise to differentiated tissues[1,2] although normal organogenesis has not been observed to progress beyond the early somite stage *in utero*[3]. Birth of live parthenogenetic rabbits has been reported[4–8], and Beatty[9] has estimated that 1 in 200 embryos induced under similar conditions can be expected to reach term. There is, however, some doubt about the validity of this early work since subsequent attempts to repeat it have failed to obtain development beyond the blastocyst stage[10,11]. Mouse parthenogenones develop to the egg cylinder stage but only one 8-somite embryo has so far been obtained[3,12]. We have examined the postimplantation development of diploid mouse parthenogenones and report that a high proportion progressed to somite embryos when blastocysts were transferred to pseudopregnant recipients. Recipients were ovariectomised following embryo transfer and their pregnant state maintained with exogenous hormones. Two apparently normal forelimb-bud stage embryos with a beating heart and yolk sac circulation were obtained. Other embryos isolated from the same recipient showed disorganised development, and a twin conceptus was obtained from a further recipient.

Cumulus masses from the oviducts of 8–12-week old (C57BL × CBA)F_1 hybrid mice were released at about 18 h after the HCG injection for superovulation into modified Krebs-Ringer bicarbonate embryo culture medium[13] containing 4 mg ml^{-1} bovine serum albumin but lacking calcium and magnesium salts (M.A.H.S. and M.H.K., in preparation). After 5–6 h adherent cumulus cells were removed with hyaluronidase, and the eggs examined to determine the activation frequency and types of parthenogenones induced (Table 1). Activated eggs were separated into their different types, transferred to normal embryo culture medium[13] and retained in culture for a further 90 h. By this time 91 out of 178 of the presumptive diploid[14,15] 2-pronuclear embryos had developed to the expanded blastocyst stage. Blastocysts were transferred to a single uterine horn of d 3 pseudopregnant recipients previously mated to proven sterile vasectomised males (day of finding vaginal plug=d 1 of pseudopregnancy). The recipients were bilaterally ovariectomised immediately after transfer of blastocysts and thereafter maintained on exogenous steroid hormones. After an initial hormone-free period of 2 d, recipients were maintained for 4 d on 1 mg progesterone daily per animal, during which time blastocysts presumably enter into quiescence.

Table 1 Types of parthogenones induced in HCG+18 h group in medium lacking Ca^{2+} and Mg^{2+}

Total no. of eggs examined	Activated eggs (%) 1 pronucleus +2PB*	2 pronuclei without 2PB	1 pronucleus without 2PB	Immediate cleavage	Overall activation frequency (%)
731	58 (11.8)	243 (49.3)	27 (5.5)	165 (33.5)	67.4

*Second polar body.

Implantation was initiated by injecting 20 ng oestradiol along with progesterone for 3 d, and thereafter pregnancy maintained with 8 ng oestradiol and 1.6 mg progesterone daily per female.

Implantation began about 24 h after the initial injection of oestradiol, which is equivalent to about d 5 of pregnancy, and recipients were killed at intervals between the 6th and 11th day of "pregnancy". About 80% of the transferred blastocysts implanted (Table 2), and all implantation sites up to d 9 were immediately fixed in Bouins solution and examined histologically. After d 8 of "pregnancy", embryos were dissected from the uterus and examined under a dissecting microscope. The two most advanced embryos obtained were at the forelimb-bud stage on the 11th d of "pregnancy", both had about 25 somites and were apparently healthy with beating hearts and a good yolk sac circulation at the time of isolation from the uterus. These embryos were later fixed for histology (Fig. 1*a* and *b*).

Table 2 Implantation rate and development of diploid (2 pronuclear) parthogenetic blastocysts in ovariectomised recipients given exogenous steroids

Day of "pregnancy" at autopsy	Total no. of recipients	Total blastocysts transferred	Total implantations	Total embryos	Approximate stage of development
6	1	3	3	1	Early egg-cylinder
7	2	14	10	3	
8	1	13	10	4	Advanced egg-cylinder
10	3	15	12	2	4-8 somite embryos
11	2	9	8	5	25 somite embryos (× 2) Disorganised embryos (× 2) Twin conceptus, one with 10 somites

Several factors may account for the improved results reported here compared with the only other comprehensive study on postimplantation mouse parthenogenetic development[3,12]. The mode of activation of eggs in Ca^{2+}/Mg^{2+}-free medium has not been used before and may be less traumatic than electrical stimulation of eggs *in vivo*[3] or hyaluronidase activation *in vitro*[16,17]. The choice of the F_1 hybrid where one-cell eggs develop to the blastocyst stage *in vitro*[15,18] is unusual since embryos from other strains do not normally progress beyond the 2-cell stage *in vitro*. The use of ovariectomised recipients may permit a better synchrony between blastocysts and the uterus before implantation commences. Blastocysts in this instance were allowed to enter into quiescence before implantation was stimulated. An increase in cell number occurs as embryos enter into quiescence[18] and would approximately double the number of cells in blastocysts before implantation.

Parthenogenetic blastocysts are similar to normal fertilised blastocysts when examined ultrastructurally[19]. Their cells can also differentiate into a variety of cell types in ectopic sites[1,2]. In the present study 35% of implanted embryos developed into normal egg cylinders, and 25% developed to somite-stage embryos.

The present study indicates that non-fertilised parthenogenetic eggs are capable of apparently normal development to a very advanced state in the absence of any genetic or extragenetic contribution from sperm. Since a greater number of embryos develop normally after fertilisation, the

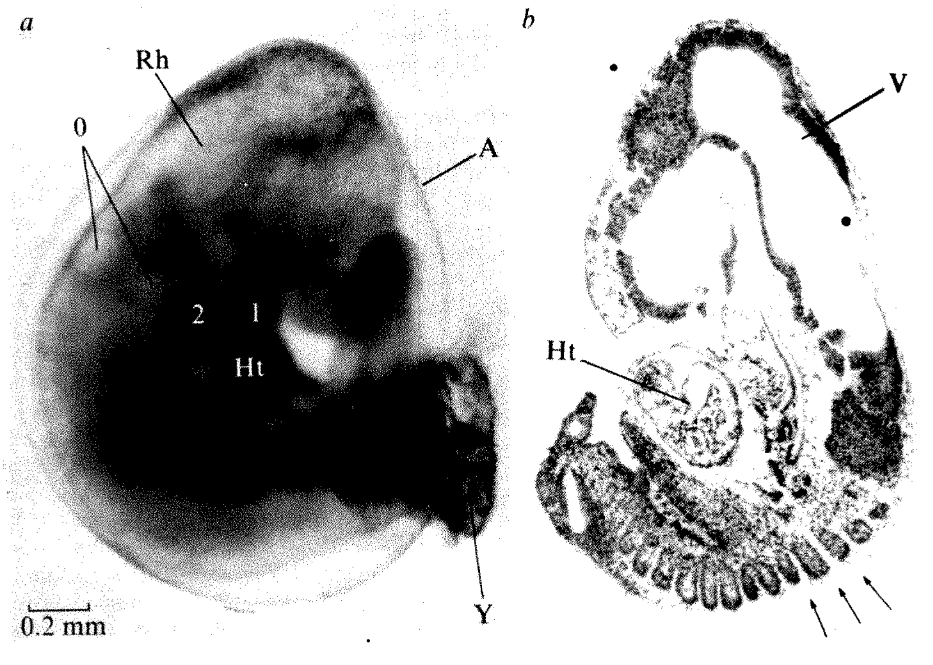

Fig. 1 *a*, Parthenogenetic mouse embryo with about 25 somites photographed within its amnion, unfixed preparation. *b*, Near sagittal section of embryo in *a* showing large number of well formed somites (arrowed). Embryo fixed in Bouins about 4 h after isolation from the uterus. Scale bar represents 0.2 mm; 1 and 2, first and second branchial bars; O, otic vesicle; Ht, heart; Rh, rhombencephalon with 4th ventricle; A, amnion; Y, yolk sac; V, 4th ventricle.

role of sperm is probably to prevent imbalance in the genetic constitution of the zygote. In the present study 60–70% of the embryos which successfully implanted died shortly thereafter, leaving about 25% to develop normally to somite embryos. In some embryos anomalous development may manifest itself in disorganisation of the embryo such that individual cells remain viable but are unable to form into tissues and organs. Survival of parthenogenones to term will depend on the extent of genetic imbalance in individual embryos which may affect development during critical stages of organogenesis.

Parthenogenetic embryos will be valuable to determine the developmental potential of genetically dissimilar embryos. The role of sperm in the activation of unfertilised eggs can be studied by morphological, genetical and biochemical comparison between fertilised and different types of parthenogenetic embryos. It may be possible to establish haploid and diploid cell lines from unfertilised parthenogenones. This would facilitate biochemical studies on cell surface constituents and their role in histotypic contact and cellular interactions. The disorganisation of some postimplantation parthenogenones probably reflects abnormalities in their cell surface properties which may be analysed by comparisons with the cells of normal embryos.

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Antigenic deletion and malignant enhancement induced in lymphoma cells by passage through X-irradiated hosts

WE report here a new system by which lymphoma cells are induced to delete strong, virus-associated membrane antigens and, as a result, considerably increase their capacity for transplantation and metastasis. This system of inducing antigenic changes in tumour cells is interesting because it involves X-irradiation of the hosts, which is used widely in the treatment of human cancer.

We used lymphoma cells which originated in W/Fu rats injected at birth with Gross leukaemia virus (GLV)[1]. The cells were obtained either fresh from rat thymomas or from our established LT_1 lymphoma tissue culture line that permanently replicates GLV[2]. As a corollary of reproducing the virus, both kinds of lymphoma cells express strong murine leukaemia virus (MuLV) surface antigens (Fig. 2) and are consistently rejected when transplanted into normal, adult, syngeneic W/Fu rats. In rats given 300–350 R total body X irradiation, however, lymphoma cells transplanted within 24 h subcutaneously or intraperitoneally (Table 1) grew progressively at the site of the graft, spread to distant sites occasionally and eventually caused the death of the hosts. Lymphoma cells from such tumours growing in X-irradiated rats were then transplanted into normal, adult, syngeneic recipients, and in contrast to lymphoma cells of thymomas and of tissue cultures (controls, Table 1) were not rejected but grew into large tumours that metastasised widely (Fig. 1) and killed the recipients. Thereafter, such lymphoma cells became serially transplantable in all syngeneic, normal, adult rats, consistently resulting in voluminous tumours, metastases and death.

As described before[1-2], thymoma cells and LT_1 tissue culture cells show GLV C-type particles at the plasma membrane and exhibit positive membrane (MF) and cytoplasmic (CF) immunofluorescence when stained with anti-MuLV serum in indirect immunofluorescence. The membrane fluorescence is ring-like, around the entire cellular circumference and is apparent on almost all cells (Fig. 2*b*), usually brighter on the cells from the culture than on those from thymomas. Lymphoma cells from tumours growing in the X-irradiated rats, however, exhibited different patterns of fluorescence. Only 10–20% of cells showed the

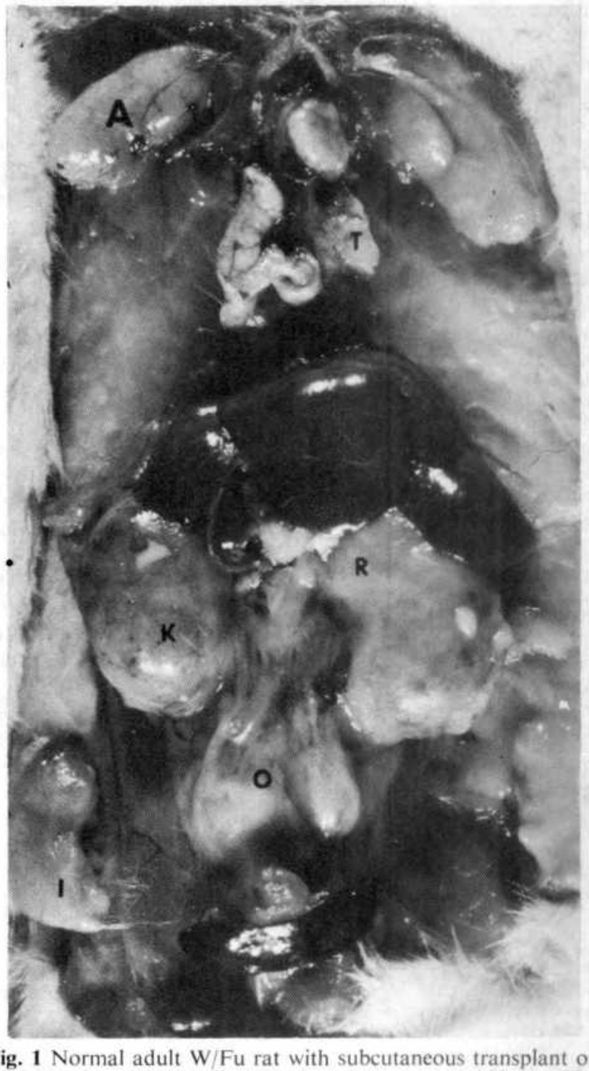

Fig. 1 Normal adult W/Fu rat with subcutaneous transplant of MuLV− lymphoma cells. Massive metastases to kidneys (K), axillary (A), parathymic (T), pararenal (R), para-aortic (O) and inguinal (I) lymph nodes.

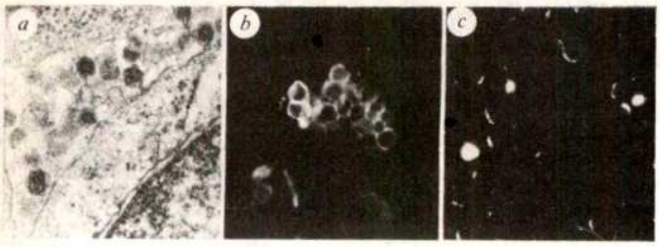

Fig. 2 *a*, MuLV+ lymphoma cell showing GLV particles at plasma membrane; *b*, MuLV+ lymphoma cell with ring-like membrane immunofluorescence; *c*, MuLV+ lymphoma cell with cap-like membrane immunofluorescence after modulation.

ring-like membrane fluorescence, while about 60% displayed capping of fluorescence (Fig. 2*a*) and 20% of the cells had become entirely negative. Tumours examined at longer intervals after transplantation in X-irradiated rats showed a progressive increase in the number of capped and negative cells with a proportionate decrease in the number of cells with positive ring-like fluorescence. Lymphoma cells of recipients that died less than 50 d after transplantation did not show loss of antigenicity as expressed by conversion from MuLV+ to MuLV− in the membrane immunofluorescence assay. Table 2 indicates that a minimum time interval was essential for the antigenic conversion to occur. As the tumours became serially transplantable to new generations of normal adult rats all lymphoma cells became permanently negative in both membrane and cytoplasmic fluorescence (Table 1).

Examined under the electron microscope, the transplantable lymphoma cells appeared devoid of both mature and immature virus particles. To investigate the apparent loss of MuLV antigens further, cytotoxicity assays were carried out using serum of rats that had rejected MuLV-positive lymphoma cells against MuLV-negative lymphoma cells as target cells and MuLV-positive lymphoma cells as controls. The results, expressed as percentages of killed cells, again confirmed the lack of MuLV antigenic expression on the transplantable lymphoma cells. As reported when antigenic conversion was induced by other means[3-5], in our experiments the loss of surface MuLV antigens was consistently accompanied by increased malignancy of lymphoma cells expressed by serial transplantability and capacity for widespread metastases (Fig. 1).

The titres of serum anti-MuLV antibodies were determined in all recipient rats by cytoplasmic indirect immunofluorescence using standard LT_1 cells as previously described[3]. The results (Table 1) unexpectedly showed higher anti-MuLV antibody titres (up to 1 : 4,096) in the normal recipients grafted with MuLV− cells that grew into progressive tumours than in those grafted with MuLV+ cells that were rejected. This might be explained by assuming that a small amount of MuLV+ cells, insufficient to induce tumour rejection but able to elicit antibody formation, was still present in the long-lasting, voluminous MuLV− tumours.

In our experiments, lymphoma cells acquired the capacity for transplantation and metastasis, consequent on the loss of their membrane viral antigens, which occurred during a passage through X-irradiated hosts. The new population of MuLV− cells may have emerged by immunoselection of nonantigenic, less vulnerable cells that were either pre-existant in the general population or appeared as a result of antigenic modulation. Defined as the phenotypic suppression of a surface antigen, occurring as a result of the cell's exposure to a specific antibody in the absence of complement[6], antigenic modulation has been reported in relation to various virus-associated[3,6-11] and histocompatibility[11-13] antigens. We have reported the antigenic deletion of MuLV antigens on lymphoma cells passaged through rats that have been rendered specifically unresponsive to these antigens at birth[3-5] as well as loss and recovery of MuLV antigens by *in vivo* and *in vitro* cultivation[9]. The importance of such phenomena lies in their possible relation to the mechanisms by which allografts and tumour cells can be unrecognised and escape immunological destruction[14-15].

In the work reported here, we are inclined to believe that the deletion of membrane viral antigens is the result of antigenic modulation, based on evidence from immunofluorescence that the membrane alterations occurred stepwise (patching-capping deletion), as well as on evidence from preliminary experiments (to be reported later), showing that the antigenic changes were reversible *in vitro*.

Table 2 Antigenicity and transplantability of lymphoma cells in relation to time after irradiation

Time after* irradiation (d)	MIF†		Transplantation‡	
	+	−	T/TR	%
17– 20	4	0	0/4	0
21– 40	11	3	2/14	14
48– 61	2	2	2/4	50
63–102	0	13	13/13	100

*Time interval between irradiation/transplantation and assays.
†Membrane immunofluorescence: no. of rats with + or −MIF.
‡T/TR = permanent tumours/transplanted tumours.

The occurrence of cellular antigenic modulation after irradiation might be related to a special state of immune imbalance created by the effect of X-rays. T lymphocytes are more susceptible to radiation effects than B lymphocytes[16-18] and one result of X-irradiation is the creation of a temporary imbalance between the two major compartments of lymphoid cells. It is also possible that B cells are increased in the general circulation, not only in relative values but also in absolute amounts in compensation for the depletion of T cells or as a result of T-suppressor-cell destruction.

Table 1 Serial subcutaneous transplantation of GLV lymphoma from X-irradiated rats

Transplant generation	Treatment	Tumours*	Metastases†	Antigens‡	Antibodies§
1	300–350R	7/9 (77)	6/9 (66)	+ − −	144(8–512)
2	None	18/21(85)	12/21(57)	− − −	684(32–4,096)
3	None	20/21(95)	20/21(95)	− − −	72(61–256)
4	None	18/20(90)	18/20(90)	− − −	25 (4–64)
5	None	14/14(100)	14/14(100)	− − −	
Controls	None	0/23 (0)	0/23 (0)	+ + +	83(32–128)

In parallel experiments GLV lymphoma cells were injected intraperitoneally in 24 irradiated and 14 non-irradiated control rats. Results were similar to those shown here.
*No. of rats with tumours at the site of subcutaneous injection/no. of rats transplanted. Percentages are given in parentheses.
†No. of rats with distant metastases/no. of rats transplanted. Percentages are given in parentheses.
‡Membrane GLV antigens in indirect immunofluorescence (each symbol represents 33% of rats).
§Titres of serum anti-MuLV antibodies determined by cytoplasmic indirect immunofluorescence on standard LT_1 tissue culture GLV-producing cells, as previously described[3]. Figures are means with the ranges in parentheses.

Antibody stimulation of tumour growth has been reported in T-cell depleted animals[19], and an excess of antibody in irradiated, B-cell-stimulated, recipients might explain the immune pressures that induce antigenic modulation.

In man, postoperative irradiation is used and credited with prevention of recurrent and metastatic tumours. But in the light of modern concepts of the role of immune surveillance in the control of tumours, the effect of X irradiation, one of the strongest immunosuppressants, has been reviewed critically. Retrospective[20] and prospective[21] studies of patients with breast cancer treated by radical mastectomy with or without postoperative irradiation showed marked increases in the number of lung and skin metastases in the former group, suggesting that radiotherapy, while effective in the control of local recurrences, might induce an increase in the number of distant metastases.

Whereas such adverse results are not entirely surprising in view of the immunodepressant effect of X irradiation, the mechanisms of its activity are not clear. Our experiments, demonstrating changes of lymphoma cells induced in X-irradiated hosts as a result of immunological imbalance, suggest a possible mechanism.

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Suppressor T-cell inactivation of a helper T-cell factor

THE ability of a subpopulation of T cells to inhibit both cellular and humoral responses is well documented[1,2]. These regulatory or suppressor T cells have been produced by both antigen-specific and nonspecific stimuli, and both antigen-specific and nonspecific suppressive mechanisms have been suggested. In various studies T cells, B cells and macrophages have all been implicated as the primary target of the suppressor cells. We[3,4] and others[5,6] have reported the nonspecific activation of both helper and suppressor murine T cells by concanavalin A (con A). The helper cell produces a nonantigen-specific mediator (NSM), of molecular weight 35,000, which can substitute for antigen-specific helper cells in the *in vitro* plaque-forming-cell (PFC) response of murine B cells to the antigen, sheep red blood cells (SRBC)[3,4]. We report here the inhibition of this response by con A-activated suppressor cells and present evidence that the mode of action of the suppressors in this system involves the inactivation of the helper factor, NSM, and not a direct effect on the responding B cells.

We initially observed that in cultures containing B cells, SRBC and NSM the anti-SRBC PFC response was severely suppressed by the addition of small numbers of spleen cells which had been activated by 48 h of culture with Con A. Extensive titrations of both the helper factor and the suppressor cells indicated a competitive relationship between the two. The results of a typical experiment are shown in Fig. 1. B cells cultured alone with SRBC produced no anti-SRBC PFC. An increasing PFC response was obtained with the addition of increasing amounts of NSM until saturation was reached. The addition of various numbers of suppressor cells to these cultures generated a series of titration lines roughly parallel to the control titration, but shifted so that the amount of NSM necessary to achieve a given response increased with the number of suppressors.

An apparent competition between con A-activated helper and suppressor T cells was originally reported by Scavulli and Dutton[7]. Because we used the cell-free helper factor, NSM, the possibility of a direct interaction between the helper and suppressor T cells seemed to be eliminated as an explanation for the competitive effect. Our results suggested instead either that con A-activated suppressor cells can

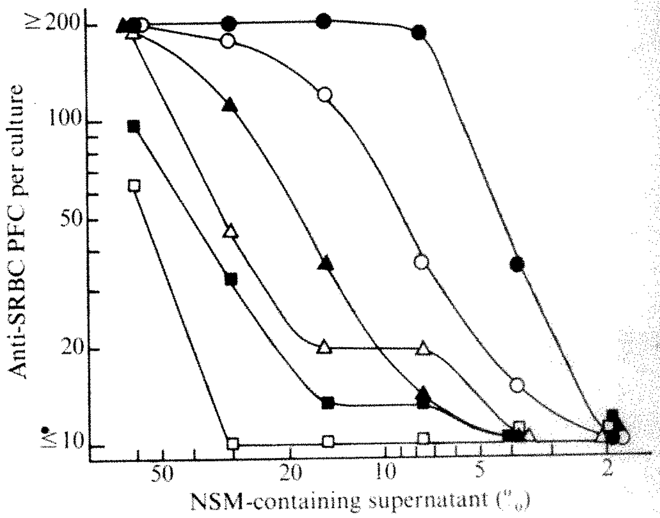

Fig. 1 Competition between NSM and suppressor cells. B cells were prepared from the spleens of BDF$_1$ mice (Jackson Laboratories) as previously described[3,4] using both an *in vivo* treatment with anti-thymocyte serum (Microbiological Associates) and an *in vitro* treatment with specific anti-T-cell serum and complement. Microcultures were prepared by modifications[3,4] of the methods of Mishell and Dutton[16]. Each culture well contained 2×10^5 B cells and 0.01% SRBC as the antigen in 0.1 ml of either medium or medium containing the various proportions indicated of an NSM-containing, 24-h supernatant from normal spleen cells cultured at 10^7 per ml with con A (4 μg ml^{-1})[3,4]; Residual con A had been removed from the supernatant by absorption with Sephadex G-200[3,4]. In addition microcultures contained either 0 (●), 7.5×10^2 (○), 1.5×10^3 (▲), 3.0×10^3 (△), 6.0×10^3 (■), or 1.2×10^4 (□) suppressor cells produced by culturing normal spleen cells for 48 h at 10^7 per ml with con A (4 μg ml^{-1}). After 4 d individual cultures were assayed for anti-SRBC PFC. Each point represents the average of the responses assayed in 12 identical cultures. In the absence of NSM all responses were less than 10 PFC per culture.

interact directly with NSM, or that they compete with NSM in their effects on B cells.

The possibility of a direct suppressive effect on B cells was eliminated in a second group of experiments. Several workers[8–12] had shown that B cells responding to SRBC were induced by the antigen to undergo at least one round of division in the absence of helper factors. Helper cells or factors were important in the response only after 24–48 h, when their addition was essential for the continued proliferation and differentiation of the responding B-cell clones to the point of antibody secretion. We looked at the effect of suppressor cells on this response when NSM was not added to cultures until 24 h after the initiation of the response by antigen (Fig. 2). The responses of control cultures were similar whether NSM was added at zero time or at 24 h. The results, however, clearly showed that the presence of suppressor cells throughout the culture period was not a sufficient condition for suppression, for suppression was not seen if the addition of NSM was delayed 24 h. This result eliminated the possibility of a direct inhibitory effect of the suppressor cells on B cells.

There are several possible explanations for the requirement that NSM must be present from the beginning of the response in order for suppression to be observed. The possibility that we explored further was that, because B cells do not develop the requirement for NSM until at least 24 h into the response, the presence of suppressor cells and NSM together during this period might lead to NSM inactivation. When added at 24 h, the NSM might interact with the then receptive B cells before its inactivation by suppressor cells.

To test the possibility that suppressor cells act directly on NSM we preincubated concentrated aliquots of NSM for 24 h alone or with various concentrations of suppressor cells. The cells were then removed by centrifugation, and the supernatants tested for NSM activity on B cells which had been cultured for 24 h with SRBC. As Fig. 3 shows, the NSM preparation preincubated alone produced a normal anti-SRBC response, whereas preincubation with increasing numbers of suppressor cells yielded successively less active supernatants. It should be noted that the ratios of suppressor cells to NSM in the preincubation step were similar to those which produced suppression in the experiments reported in Figs 1 and 2. These results indicated that the full activity of the suppressor cells could be accounted for by the direct elimination of NSM activity.

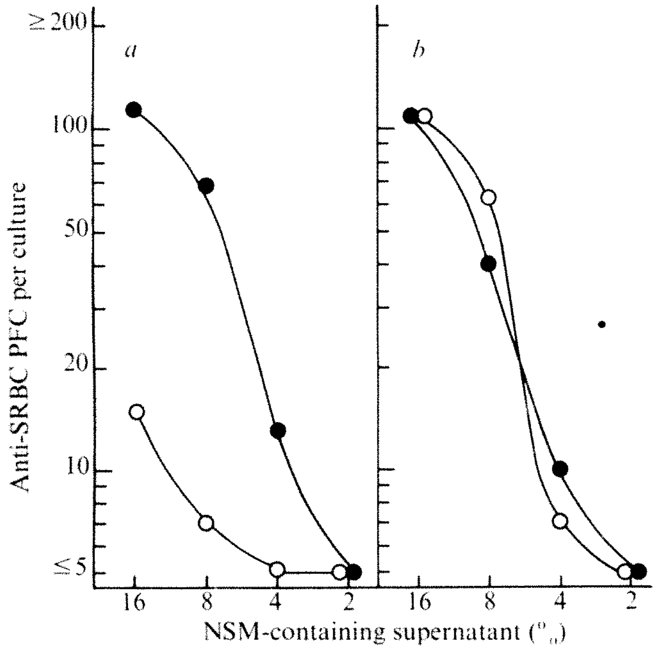

Fig. 2 Loss of suppression with the delayed addition of NSM. NSM containing supernatant, suppressor cells and B-cell cultures containing SRBC were prepared as in Fig. 1. *a*, A group of B-cell cultures received various proportions of NSM supernatant at time zero (●). A second identical group received in addition 2×10^3 suppressor cells at time zero (○). *b*, Same as (*a*) but the addition of NSM was delayed until 24 h. All cultures were assayed for anti-SRBC PFC on day 4. The results are pooled from two identical experiments. Each point is the average response of 24 identical cultures. In the absence of NSM all responses were less than 5 PFC per culture.

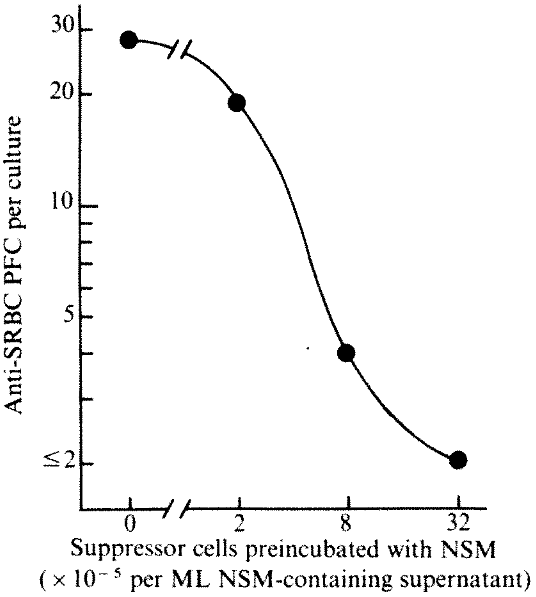

Fig. 3 Inactivation of NSM by preincubation with suppressor cells. Neat NSM-containing supernatant, prepared as in Fig. 1 was preincubated for 24 h at 37 °C either alone or with various concentrations of suppressor cells, prepared as in Fig. 1. B-cell cultures containing SRBC were prepared as in Fig. 1. After 24 h the various preincubated supernatants from which cells had been removed by centrifugation were added to the B-cell cultures to a final concentration of 10%. After an additional 3 d all cultures were assayed for anti-SRBC PFC. The results of four similar experiments were combined. Each point represents the average response of from 36 to 48 individual cultures. In the absence of NSM the response was less than 2 PFC per culture.

Several additional control experiments were performed. For example, the cell responsible for the NSM inactivation was shown to be eliminated by treatment with anti-T serum and complement whether before or after con A activation (data not shown). Typical results of a second control experiment are shown in Fig. 4. As expected, NSM preincubated with excess suppressor cells lost all activity. Preincubation of NSM with the same number of normal spleen cells, however, resulted in no loss of NSM activity, demonstrating the need for con A activation to produce the suppressor cells. In addition, the loss of NSM activity was not due to its masking by an inhibitory factor produced by the suppressor cells or to the conversion of NSM to an inhibitory factor, for either medium or NSM preincubated with suppressor cells had no effect on the activity of fresh NSM. These latter results also suggest that the loss of NSM activity was not through the action of a soluble degradative enzyme, but rather required direct interaction of the NSM with the suppressor cells. We are attempting to distinguish whether this interaction resulted simply in the stoichiometric absorption of NSM by a suppressor cell receptor or in its destruction at the cell surface.

In summary, our results indicate that the action of con A-activated suppressor cells on the response of B cells to the antigen, SRBC, and the helper factor, NSM, is through the inactivation of the NSM rather than a direct effect on B cells. They suggest that one mode of action of regulatory T cells is the control of the level of immunologically active mediators.

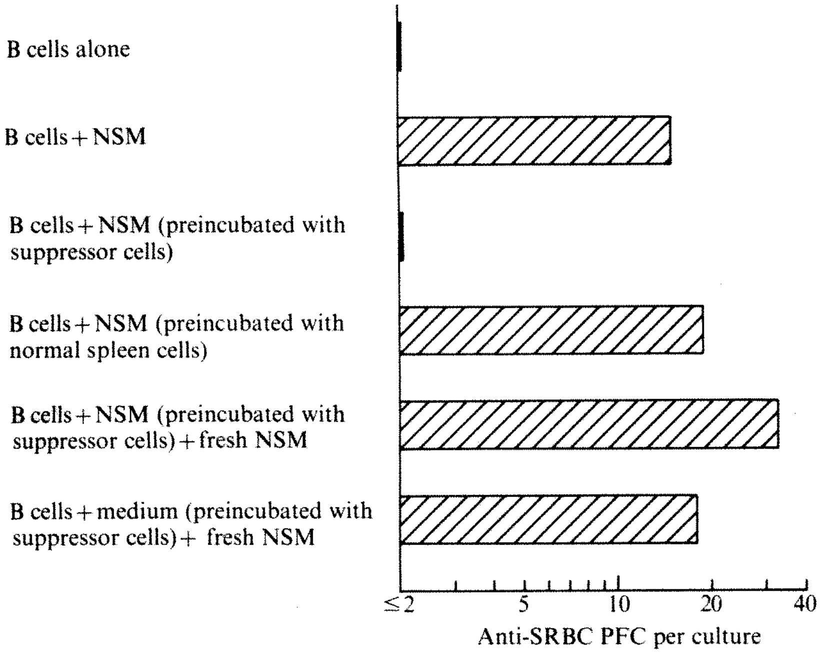

Fig. 4 Need for con A activation of the suppressor cells and the lack of the production of a soluble suppressive activity. NSM-containing supernatant, prepared as in Fig. 1, was preincubated for 24 h at 37 °C either alone or with 8×10^3 suppressor cells per ml or with 8×10^3 normal spleen cells per ml. In addition normal medium was preincubated with 8×10^3 suppressor cells per ml in the same conditions. B-cell cultures containing SRBC were prepared as in Fig. 1. After 24 h the various supernatants prepared as above were added to the B-cell cultures to a final concentration of 10%. Some cultures received in addition fresh NSM supernatant to final concentration of 10%. After a further 3 d all cultures were assayed for anti-SRBC PFC. Each bar represents the average response of twelve identical cultures.

Our results are somewhat at odds with those of Pierce *et al.*[13,14], who have proposed that the activity of con A-activated suppressor cells is accounted for by the production of a factor, indistinguishable from macrophage migration inhibitory factor, whose mode of action is to cause normal macrophages to become suppressive. Perhaps macrophages stimulated in this manner also develop the ability to inactivate helper factors. Our results also raise a question concerning the clearly demonstrated ability of con A-activated suppressor cells to inhibit responses to thymus-independent antigens[3,4,15]. Because these responses presumably do not involve helper factors, the mechanism of suppression is unclear. A direct interaction between suppressor cells and this special type of antigen is an intriguing possibility; however, alternative suppressive mechanisms involving direct effects on B cells are also possible.

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Androgen-modified expression compared with Y linkage of male specific antigen

SEVERAL recent results have led to a widely held view that the male specific antigen (MSA) is coded for by a structural gene on the Y chromosome, and because of this it is frequently termed H-Y (refs 1 and 2). Although a good case has been put forward for this conclusion, there are some data that conflict with this view and some arguments against the notion. These are presented here as a reminder that, as is so often the case with biological experiments, the Scottish verdict "not proven" holds.

Evidence in favour of a Y-linked structural locus for the MSA has led to a hypothesis that the Y-linked gene(s) required for testis development and the MSA structural locus are one and the same[3,4]. It is theorised that MSA acts as a partially diffusible hormone able to induce male gonal formation in coated XX cells and evidence for this mechanism has been presented for bovine freemartins[5]. But strong evidence has been presented against Y linkage of the MSA[6]. This is the observation that XX, *Sxr* (sex-reversed) males have MSA. In spite of cytogenetic studies involving various new staining techniques, there is no evidence for translocation Y material in XX, *Sxr* males[7,8] and the presence of MSA in XX, *Sxr* males is incompatible with Y linkage of the structural gene for MSA. Evidence for the presence of MSA in phenotypically female, XY, *Tfm* mice has been presented as evidence that the MSA is not a concomitant of maleness *per se* and, therefore, must be Y-linked. An alternative hypothesis states that the structural locus for the MSA is autosomal, that its expression is triggered by a male determinant normally controlled by a portion of the Y chromosome, and that apparent Y-linked allelic differences in the MSA are the reflection of altered levels of this Y-chromosome factor, due to allelic forms of the Y-chromosomal locus. *Sxr* could be a constitutive mutant of the autosomal locus normally induced by the Y-chromosome factor. According to this hypothesis, the evidence that MSA is present in phenotypically female *Tfm*/Y mice[9], which have high circulating levels of testosterone, provides the limitation that, if the

Table 1 Summary of Y-linked 'fertility' data

Characteristic	Strains compared	Evidence for Y linkage	Ref.
Mean sperm dimensions	Strains selected for large virus small body size	Paternal cytoplasmic effect or Y-linked effect	45
% Morphologically abnormal sperm	C57B1 (high) against CBA (low)	Portion of effect Y-linked	46
% Morphologically abnormal sperm	KE (high) against KP (low)	Portion of effect Y-linked	47
% Morphologically abnormal sperm (with fertility inversely correlated)	KE (high) against CBA (low)	Portion of effect Y-linked	48
Male behavioural differences	PHH against PHL	Y-linkage accounts for all the difference	49
Male aggression	DBA/1/Bg against CBA/FaCam (low)	Y-linkage or maternal effect	50
Adult testes size	SF (high) against CBA/FaCam (low)	41 % Explained by Y linkage	51

MSA is mediated by testosterone, the hormone does not act through a target organ mechanism limited to males (the cytosol testosterone receptor thought to be altered in *Tfm*[10,11] is present in females as well as males[3,12]) This mechanism would already seem unlikely because of the presence of MSA on epidermal cells, where it is detected by skin grafting, and other tissues which are not primary testosterone targets

Two other aspects of research on the MSA may be responsible for part of the confusion. First, the work on MSA has been performed by skin grafting; only recently has serological detection of the MSA been possible[13]. The antigen detected with mouse sera cross reacts with cells from other mammals studied, including man[14], and even amphibia and birds, where the female heterogametic sex has the antigen[15]. Grafting techniques, however, have not established a MSA in man[16,17] or amphibia[18] but the lack of inbred strains in these species may be the problem, that is, it is not possible to make male-to-female grafts between individuals with otherwise identical genotypes. Immunological cross reactions between such widely divergent species are likely to be due to something other than a protein, for example, a carbohydrate or a steroid, or a protein whose function is extremely stable in evolution, such as histone IV. If one is to argue that the evolutionary conservation of the MSA reflect a conserved function, there is the problem that its function must be related to female sex determination in WW, WZ species. If the MSA is a protein coded for by the heterogametic sex chromosome, it might be simpler to postulate that it is a protein involved in the synthesis or packaging of the unique, heterogametic tandemly repeated DNA[19]. Second, differences in the MSA detected by skin grafting may not reflect different alleles but different loci, that is, there could be a MSA with a Y-linked structural gene and other MSA(s) which are autosome-linked and androgen-dependent.

The original evidence that the MSA locus was on the Y chromosome was of two sorts. First, teratoma lines maintained by *in vivo* passage have a tendency to lose the Y chromosome and apparently lose the MSA concurrently[20]. But the reported loss of the MSA in teratomas which had lost the Y chromosome depended on very few grafts; some Y-containing cells persisted in the accepted grafts; and the work was not confirmed[21]. Second, there are various strengths of the MSA, originally detected by skin grafting[22–24] and possibly found serologically[13], which are Y-linked Other workers, however, have not been able to reproduce some of the results suggesing alternative forms of a Y-linked antigen[25–26]. More recently, a dose relationship between the number of Y chromosomes and the amount of seriologically detected MSA in humans has been used to argue for Y-linkage of the MSA[27]. Studies on humans have also shown MSA in XX males with no detectable XY cell line[28], a situation analogous to *Sxr*, XX mice. Thus, the data are also highly compatible with the interpretation that MSA is a concomitant of maleness which can occur occasionally in the absence of a Y chromosome, for example, polled in goats[29], as femaleness can occasionally ccur in the presence of a Y chromosome[30]

There is a variety of evidence to suggest that the MSA depends on androgens for its expression. Neonatal orchiectomy of C57B1/6 males leads to prolonged, or even permanent, survial of the adults' skin on females of the same strain[31,32], while neonatal male skin grafts allowed to develop after being transplanted to adult females do not reach normal adult male skin immunogenicity for secondary female recipients until after more than 100 d[33]. Furthermore, female responsiveness to male skin grafts is specifically depressed by testosterone injections started at birth[34], while skin from males pretreated with a non-functional analogue of testosterone, cyproterone acetate, has prolonged survival on females[35]. Thus, expression of the MSA is not complete in these situations, in spite of the presence of the Y chromosome. Testosterone, however, can have inhibitory effects on the immune response[36] and this could explain effects on female responsiveness.

The observation that MSA expression can be modified by *H–2* region-linked genes[37], which is also controversial[34], could be explained by a previously described *H–2*-linked effect on testosterone levels[38,39]. (The effect of *H–2* on MSA expression seems to be separate from the role of *H–2* in the specification of the MSA target cell complex for cell-mediated cytolysis[40], a role *H–2* antigens also play with hapten or virus-induced cell surface antigens[41–43].) It should be added that the anti-androgenic steroid also reduced expression of *H–3* and *H–2* antigens, as measured by the surival of skin grafts from treated males transplanted across those barriers[44]. The latter result suggests that androgens could easily play an important role in the expression of antigens not linked to the Y chromosome, including the MSA.

There are various Y-linked differences among males of different inbred strains, and most of these putative Y-linked phenotypic differences could be secondary to levels of androgen (Table 1). The difference which should be most clearly related to androgen levels is that of testes size; 41% of the difference between CBA/FaCa and SF strains was Y-linked[51]. Thus, if the original data suggesting Y-linked alleles for the MSA are correct, these may be explicable as secondary effects of Y-linked differences in androgen levels. The Y chromosome "switch" for male-determination is postulated to have several alternative levels of expression. The apparent double dose of Y antigen in human males with two Y chromosomes[27] is also compatible with this hypothesis

If the MSA is actually controlled by an autosomal locus but modified by androgen activity (with the latter possibly showing allelic variation controlled by the Y chromosome), the presence of MSA in phenotypically female, *Tfm*, XY mice[9] argues that the action of androgens on the MSA is not through a mechanism limited to testosterone target organs of males Testosterone seems to have important functions in female reproduction[52] and to be involved in protein synthesis in the female liver[53]. The latter function of androgen in females is mediated by microsomal membrane testosterone receptors[54] which are under hypothalmic control[55]. There are other examples of steroid hormone function which are not mediated by cytosol receptor-nuclear translocation mechanisms. There is evidence that oestrogen[56] and glucocorticoids[57–58] have direct effects on intracellular cyclic AMP levels while oestrogen releases histamine from the rat uterus[59] and progesterone mediates effects on frog oocyte differentiation which involve an effect on the outer surface of the cell membrane, without a requirement for entrance of the hormone into the cell[60]. Thus there are androgen responses which are not male, target-organ-limited, but if they are responsible for MSA expression they should not all be shared by the female. If they were, MSA should not be detectable because the antigen should be produced in the females and they would be tolerant. Rather, it is assumed that various androgen functions, some of the target organ type and some mediated by other pathways but male-limited, are "turned on" in the male by the Y-linked trigger which may have a primary effect on one autosomal locus such as the *Sxr* locus. Then it would be possible that the *Sxr* locus product is MSA[6], while its expression would normally be controlled by a Y chromosome.

There are two major arguments against this alternative hypothesis. First, there is the reported detection of antigen cross reacting with murine MSA in females of birds and reptiles where the female is the heterogametic sex[15]. There is also evidence that female-to-male grafts are rejected in chickens[61]. But this creates a theoretical dilemma: if the antigen is a protein then it has been uniquely stable in evolution when its function has changed to become female determinitive while if it is not a protein, the argument that Y-linked allelic forms demonstrate a structural locus on the Y chrososome is much more tenuous. Second, there is the apparent finding of MSA on putative XY mouse embryos at the eight-cell stage[62]. This cannot be explained by secondary effects of maleness but needs further confirmation: embryos were classed as "dead" if only one cell was lysed and in no case were more than five cells lysed. The 50% "death" of eight-cell embryos can be explained just as readily by assuming that all the embryonic cells are equally sensitive to antibody plus complement. If the probability that any given cell will be lysed is only 0.083, then 50% of the embryos will remain unaffected, while the remainder should have one or more lysed cells, the number of cells lysed per eight-cell embryo following a binominal distribution. Such a distribution predicts the presence of very few embryos with four or more dead cells. Although the actual data have not been presented, larger than expected numbers of embryos with four or more dead cells might simply reflect some degree of co-operativity, with the death of one cell rendering the adjacent cells more susceptible to lysis.

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Specific binding of a synthetic oligodeoxyribonucleotide to yeast cytochrome *c* mRNA

THE investigation of specific messenger RNAs in terms of sequence analysis and of the control of their synthesis, processing, translation and degradation is one of the major interests of modern molecular biology. These studies have been possible so far in only the few cases where a cell type is known to synthesise predominantly one species of protein. Since most mRNAs are chemically similar, differing only in size and sequence, it has been difficult to purify them by conventional methods. Given the amino acid sequence of a protein, one can use the genetic code to deduce a sequence of nucleotides in a specific region of the mRNA and then synthesise an oligodeoxyribonucleotide complementary to that region[1–4]. We have chosen to investigate the yeast iso-1 cytochrome *c* mRNA as a model system for the use of synthetic oligonucleotides as specific probes of eucaryotic mRNA structure and function. The genetics of yeast iso-1 cytochrome *c* has been studied in great detail by Sherman, Stewart and co-workers[5,6], who have determined the sequence of 44 nucleotides of the mRNA from the analysis of altered cytochromes produced by a series of double frameshift mutants. In addition, a number of well defined deletion mutants are available within this region. The pentadecadeoxyribonucleotide (15-mer) that we decided to synthesise, 5′-d(A-G-C-A-C-C-T-T-T-C-T-T-A-G-C)3′ is complementary to nucleotides 25–39 of the cytochrome *c* mRNA (Fig. 1). This sequence was chosen because it is relatively easy to synthesise, should form a very stable hybrid with mRNA, and is close to the 5′ end of the coding region of the mRNA. A known deletion mutation covers this region, yet allows the production of a functional cytochrome *c* in normal amounts[5,6], and thus provides an excellent control for hybridisation experiments. We have been able to show that the

```
      14  13  12  11  10  9   8   7   6   5   4   3   2   1   -1
A.    Leu-Thr-Ala-Gly-Lys-Lys-Ala-Ser-Gly-Ala-Lys-Phe-Glu-Thr-(met)

       40          30          20          10           1
B.    NUC ACA UCG UGG AAA GAA UCG UCU UGG CCG GAA CUU AAG UCA GUA. . .5'
           1'          11'   16           29'
C.     5'-d|AGC ACC TTT CTT AGC|AGA ACC GGC CTT GAA TTC AGT CAT. . .3'
```

Fig. 1 Amino acid sequence of yeast iso-1 cytochrome *c* (*a*), and nucleotide sequence of its mRNA (*b*,) with the complementary DNA sequence *c*. The boxed-in area represents the synthetic 15-mer.

synthetic 15-mer does indeed bind specifically to the expected region of the mRNA, and therefore should be useful as a specific probe for the study of this mRNA.

The chemical synthesis of the pentadecanucleotide was by the modified phosphotriester method of Narang and co-workers[7,8]. Recent developments in this approach to synthesis, such as the introduction of new reagents for phosphorylation and condensation, have greatly reduced the time required to synthesise oligonucleotides 12–21 nucleotides 3 long. Our synthesis proceeded in three stages. First, completely protected mononucleotide 3′ phosphates were prepared according to the procedures of Narang and co-workers[8,10] except that the methylbutyryl group was used for the protection of the amino group of deoxyguanosine[11], and the 3′ OH of the primer was protected by acetylation[12]. Secondly, the monomers were condensed into di- and tri-nucleotides as described earlier[8,10]. Finally, the five trinucleotides were assembled using mesitylenesulfonyltetrazole as a condensing reagent[13,14]. The final product was deblocked and the 15-mer purified on a Sephadex G-50 column followed by chromotography on a DEAE-cellulose column in 7 M urea with a final yield of 10 μmol.

We have confirmed the sequence of the synthetic oligonucleotide by direct sequence analysis (Fig. 2). The 15-mer was labelled with ^{32}P at the 5′-end with polynucleotide kinase[15], or at the 3′-end with terminal transferase[16]. The labelled material was partially digested with venom or spleen phosphodiesterase, and mapped by electrophoresis on Cellogel strips followed by homochromatography[15,17,18]. Each map consists of a series of spots which represent the products of partial digestion of the starting material. The mobility shift of each successive spot is characteristic of the added nucleotide[18]. In all cases the mobility shifts are as expected. Furthermore, 5′-end analysis shows that >95% of the 5′-nucleotide is A and the 3′ nucleotide is C.

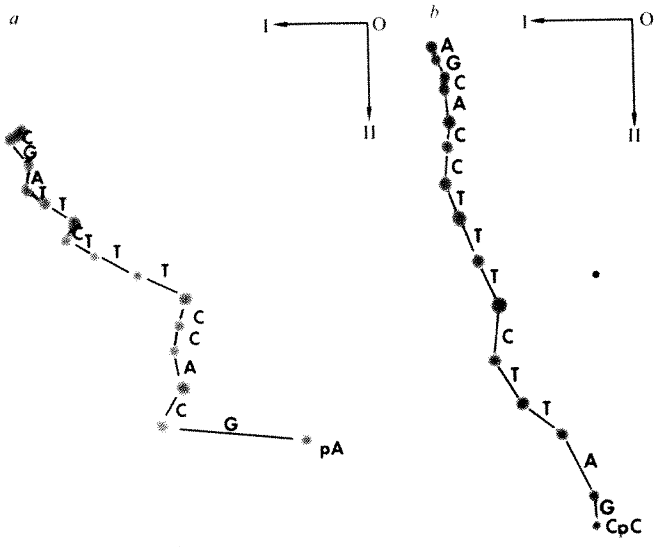

Fig. 2 *a*, Two-dimensional homochromatography of a partial venom phosphodiesterase digest of the synthetic pentadecanucleotide d(^{32}pA-G-C-A-C-C-T-T-T-C-T-T-A-G-C). Dimension I, electrophoresis on cellulose acetate strip (Cellogel, Reeve-Angel), *p*H 3.5 at 2000 V for 40 min. Dimension II, homochromatography on DEAE-cellulose plate (20 × 20 cm), with Homo-mix IV[15]. *b*, Two-dimensional homochromatography of a partial spleen phosphodiesterase digest of the 16-mer d(A-G-C-A-C-C-T-T-T-C-T-T-A-G-C)^{32}prC produced by transferase labelling with (α – ^{32}P)rCTP of synthetic 15-mer. Dimensions are as in Fig. 2*a* except that homochromatography was done on a 20 × 40-cm plate with Homo-mix III[15]. The migration of the spots is less in the electrophoresis dimension because there is an -OH group at both the 5′-end and the 3′-end of the oligonucleotides.

Hybridization of the 15-mer to the cytochrome *c* mRNA can be detected by incubating bulk mRNA with an excess of the ^{32}P-labelled oligonucleotide under the appropriate conditions, and then separating the free from the bound 15-mer by chromatography on Sephadex G-75 or by electrophoresis in a 5% polyacrylamide gel. Both methods gave the same results but the latter method is most suitable for the simultaneous analysis of a large number of samples. We found that most of the binding to mRNA (>80%) from derepressed wild type yeast (grown with aeration on ethanol) is due to specific hybridisation to iso-1 cytochrome *c* mRNA. In control experiments, the binding of 15-mer to mRNA from either repressed wild type cells (grown under nitrogen with high glucose), or from derepressed cells which carry a deletion of the complementary region, is reduced to less than 20% of the binding to mRNA from derepressed wild type cells. The amount of 15-mer bound to wild-type mRNA is in direct proportion to the amount of mRNA present (data not shown). When the amount of mRNA in a hybridisation reaction

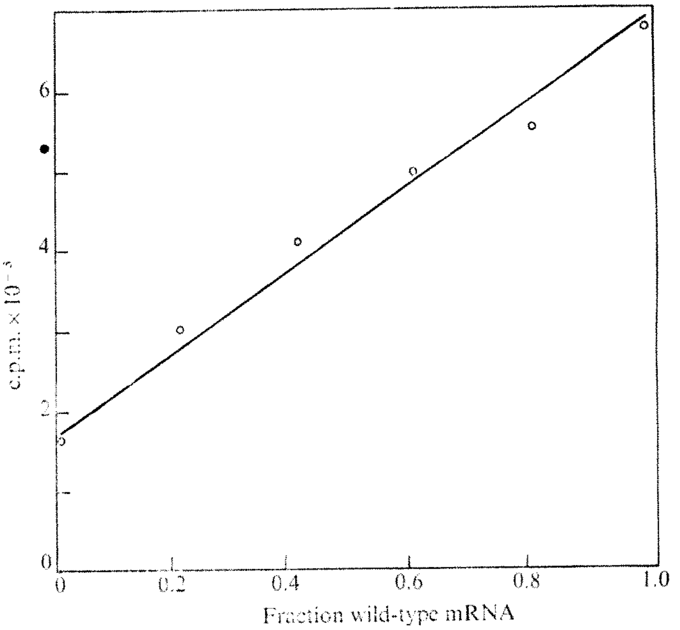

Fig. 3 Specific hybridisation between synthetic (^{32}P)15-mer and yeast cytochrome *c* mRNA. Total RNA was isolated by breaking chilled yeast cells in a Braun homogeniser in the presence of phenol, followed by centrifugation and ethanol precipitation of the aqueous layer. Electrophoretic analysis of this RNA showed very sharp ribosomal RNA bands with no evidence of any degradation products. The mRNA fraction was isolated from the total RNA on poly-U Sepharose column[2]. 1.0 pmol of (^{32}P)15-mer and 50 μg of mRNA were dissolved in 50 μl of H-buffer (0.1 M Nacl, 0.01 M Tris-Cl, *p*H7.5, 0.001 M EDTA, 0.05% SDS). The amount of mRNA from derepressed wild-type cells (stain D311-3A, *a*, *his1*, *trp2*, *lys2*) was varied from 0 to 50 μg, with each sample made up to 50 μg total with mRNA from derepressed deletion mutant cells (strain B2185, aCYC-1-183-AD, *his1*, *trp2*, *lys2*). Strains of yeast were a gift of Dr Fred Sherman. The reaction mixtures were incubated at 37 °C for 18 h, at which time 10 μl of loading dye (60% sucrose, 0.2% bromophenol blue) was added to each sample. Electrophoresis was carried out for 3 h at 3 V/cm on a 5% polyacrylamide gel (5% cross-linking with *bis*-acrylamide). All mRNA and bound primer was contained in an area within 0.5 cm from the origin, which was cut out and counted. Approximately 0.1–1.0% of the input labelled primer was bound to mRNA.

is made up to a constant amount with mRNA from deletion mutant cells, the amount of 15-mer bound increases in direct proportion to the amount of mRNA from wild type cells (Fig. 3). Therefore, the 15-mer is specifically hybridizing to the cytochrome *c* mRNA.

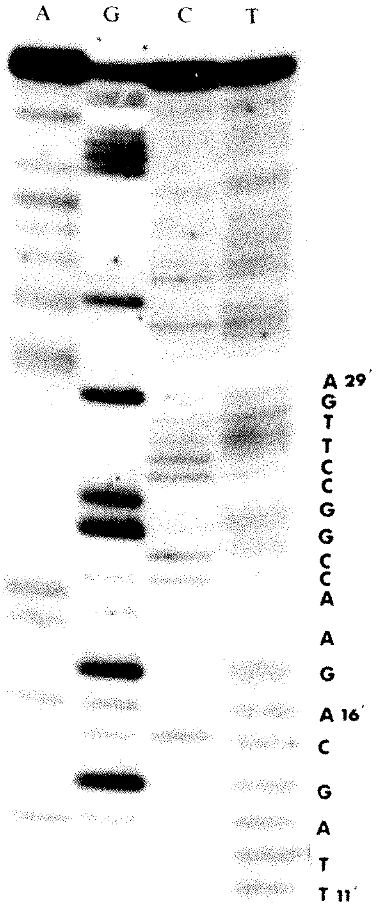

Fig. 4 Enzymatic extension of 15-mer primer hybridised to yeast cytochrome *c* mRNA. 5 mg of mRNA was incubated with 500 pmol of 5′(^{32}P)15-mer (1000 mCi μmol^{-1}) in 5.0 ml of H-buffer at 37 °C for 44 h. The primer-mRNA hybrid (1.1 × 10^6 c.p.m.) was separated from free primer on a Sephadex G-75 superfine column (2.4 × 35 cm) in 0.1 M NaCl, 0.01 M Tris-Cl pH 7.5. The 15-mer primer was extended by the incorporation of unlabelled dNTPs as follows[19]. The solution containing the primer-mRNA hybrid (11.0 ml) was adjusted to a final concentration of 25 mM HEPES buffer (pH 7.5), 1 mM $MnCl_2$, and 5 μM of all four dNTPs. *E. coli* DNA polymerase I (100 units) was added and the reaction allowed to proceed at 1.5 °C for 4 h. The NaCl concentration was adjusted to 0.3 M and 2 vol. of ethanol were added. The mixture was chilled to −20 °C for 30 min and centrifuged at 10,000 r.p.m. in an HB-4 rotor for 20 min. The pellet, which contained 99% of the radioactivity, was dissolved in 0.3 ml of 0.3 N NaOH and incubated at 37 °C for 16 h to hydrolyse the RNA. To fractionate the labelled extended primer, the sample was loaded on a 20 × 20 × 0.3 cm polyacrylamide gel (10% acrylamide with 5% cross-linking, 7 M urea, 50 mM Tris-borate, pH 8.3) and electrophoresed at 400 V until the bromophenol blue had migrated 37 cm. The gel was then autoradiographed, the labelled bands eluted, concentrated on a DEAE-cellulose column, eluted with 2 M NaCl, and ethanol precipitated. The products of the primer extension reaction were sequenced by the chemical method of Gilbert and Maxam[20]. A-specific cleavage is obtained by partial purine methylation with dimethylsulphate, preferential depurination in dilute HCl, followed by chain cleavage with alkali. G-specific cleavage is obtained by partial methylation as above, followed by chain cleavage with piperidine. C- and T-specific digestions are obtained by partial hydrazinolysis (in the presence of 1 M NaCl for a C-specific reaction) followed by piperdine treatment to break the chain. The products of these four base specific partial digestions are separated on four lanes of a polyacrylamide slab gel (20% with 3% crosslinking, 7 M urea, 50 mM Tris-borate, pH 8.3). The autoradiograph of such a gel is shown above. The sequence is deduced from the presence of specific bands on each of the four lanes.

We have sequenced a short region adjacent to the binding site of the 15-mer as further proof that the 15-mer binds to the expected region of the yeast cytochrome *c* mRNA. DNA polymerase I will extend a DNA primer on a RNA template[19] in the presence of Mn^{2+} in place of Mg^{2+}. When 5′-(^{32}P) 15-mer bound to mRNA was extended under these conditions, a series of products was obtained which were separated by polyacrylamide gel electrophoresis in the presence of 7 M urea. The length of these products as determined by comparison with single stranded DNA markers ranged from 15 to approximately 120 nucleotides, implying the presence of at least 80 nucleotides proximal to the initiation codon of this mRNA. Sequence analysis of the major bands by the new chemical technique of Gilbert and Maxam[20] has shown that the sequence of the short products is part of the sequence of the long products. The 14 nucleotide sequence which we have determined (Fig. 4), 5′-d(A-G-A-A-C-C-G-G-C-C-T-T-G-A)3′, confirms the sequence Sherman and Stewart[5] determined by genetic analysis (Fig. 1 *b* and *c*). This provides additional evidence that the synthetic 15-mer is binding specifically to the expected site on the yeast iso-1 cytochrome *c* mRNA.

We are extending our studies to determine the sequence in the regulatory region of this yeast mRNA. The use of synthetic oligonucleotides as specific probes will make it possible to study many mRNAs which could not otherwise be approached.

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Anomaly in the γ chain heterogeneity of the newborn

THE two types of γ chain in human foetal haemoglobin (HbF) are the products of nonallelic structural genes and have either glycine ($^{G}\gamma$ type) or alanine ($^{A}\gamma$ type) in position 136 (ref. 1). Although the examination of 108 cord blood samples from worldwide sources showed 102 to have a $^{G}\gamma$: $^{A}\gamma$ ratio within a relatively narrow range, one had a low ratio and five a high ratio[2]. It seemed possible that

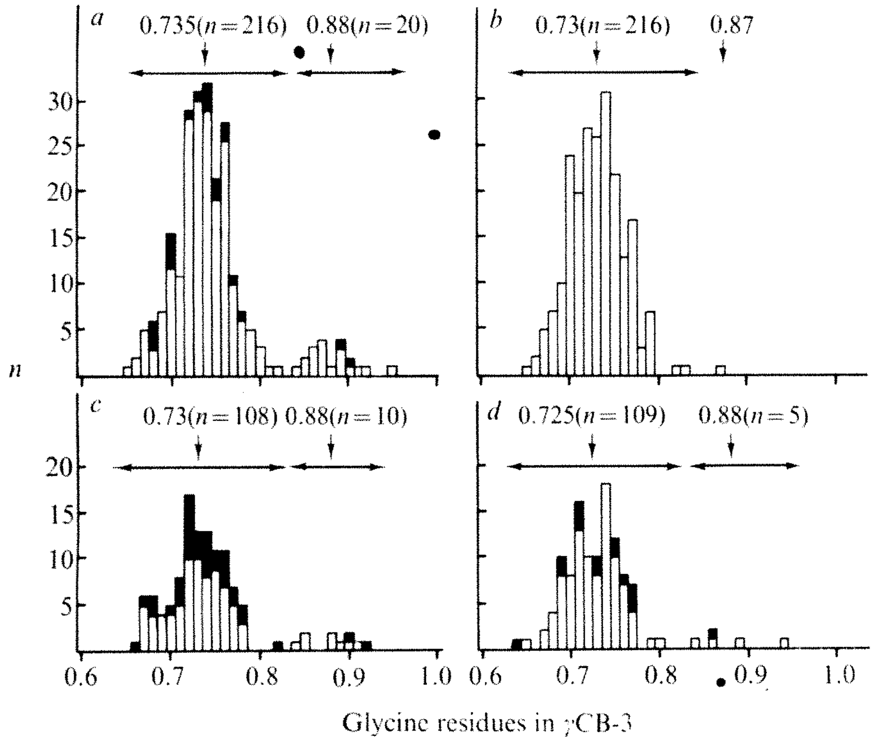

Fig. 1 Distribution of glycine residues of γCB-3 from the cord bloods of black and Caucasian newborns from different areas. Open columns denote data from newborns without haemoglobinopathy, and black columns denote data from HbS- and/or HbC-containing samples. *a*, Burke County blacks; *b*, Caucasians; *c*, Ghana blacks; *d*, Los Angeles blacks.

analytical error was responsible for these extreme values. But as we studied cord blood from normal black babies of residents from Burke County, Georgia, where an abnormal HbF (designated F_X (ref. 3)) has been detected repeatedly, we observed high ratios in further samples. The present study of almost 700 cord bloods has detected the high $^{G}\gamma : {}^{A}\gamma$ ratio in about 7.5% of samples from black newborns as compared with perhaps 0.5% from Caucasian infants.

The nonallelic γ genes are found worldwide and are not limited to specific ethnic groups or geographical areas[2]. Experimentally, the heterogeneity is best determined by cleaving the HbF with cyanogen bromide, isolating peptide γCB-3, and making an amino acid analysis for glycine and alanine according to the procedure described previously[1]. If the residues of glycine are integral at 1 or 0 and alanine at 2 or 3, the $^{G}\gamma$ or $^{A}\gamma$ chains alone are present in the HbF; a nonintegral number denotes a mixture. It is simplest to discuss results in terms of 'glycine values' rather than the $^{G}\gamma : {}^{A}\gamma$ ratio. Thus, in the initial study (2), 102 samples had glycine values between 0.65 and 0.79 residue; one was very low at 0.52 and five were above 0.8 residue.

The initial sampling of our extended study included only black children from Burke County. Subsequently, the survey was expanded to include blacks and whites from the Los Angeles area, blacks from Ghana, and whites from the Augusta area and from Malta.

That analytical error was not responsible for higher values was evident from reproducible data of 11 duplicate analyses which agreed with one exception within 0.05 residues as expected[4].

The data are summarised in Fig. 1. Clearly the anomaly manifests itself almost exclusively in black infants of whom 35 (of 468 studied) have glycine values above 0.82 residue in contrast to Caucasian infants of whom perhaps only one (of 217 studied) has the effect. The seemingly different incidence of 8.5% among Burke County as well as Ghana black babies compared with 4.4% in Los Angeles area black newborns may be attributed to more settled and less migratory communities in Burke County and Ghana compared with the more cosmopolitan sources of blacks in the Los Angeles area. The incidence of the anomaly in infants with HbS and/or HbC is 7.1% (five out of 71 infants), accords with the general incidence and suggests no relationship to an abnormal β gene.

Newborns with the anomaly are healthy babies without haematological problems. The range and average of alkali resistant haemoglobin by the Betke method[5] was not significantly different in newborns who had glycine values above or below 0.82 residue. All nine mothers and fathers of newborns with the anomaly who were studied had normal haematological indices and percentages of HbA_2 in the normal range of 1.7–3.7% (ref. 6). Two mothers and one father had sickle cell trait, one father had AC trait, and one mother had a slightly elevated level of Hb-F (Hb-F_{AD} : 4.1%).

It has been postulated that there may be as many as four structural γ chain loci (two $^{G}\gamma$ and two $^{A}\gamma$ loci (ref. 3)). Recent evidence suggests only two or three loci[7]. Several regulatory factors seem to be interspersed among the complex of β, γ and δ genes to control the production of protein by each gene[8]. Neither the nature nor the mode of action of these regulatory factors is known. Perhaps, the anomaly exerts its action either by increasing the production of the gene(s) for the $^{G}\gamma$ chains or vice versa by decreasing the activity of gene(s) for the $^{A}\gamma$ chain. Incomplete investigations suggest that the anomaly is hereditary.

As the HbF decreases postnatally until only traces of HbF remain in the adult, the glycine value also changes from approximately 0.73 in the infant to 0.4 (range about 0.25–0.55) in the adult[9]. Does a high glycine value at birth remain high or does it change to the adult range? Conversely, can a 'normal' glycine value of about 0.7 at birth remain unchanged? It has indeed been observed in a newborn with sickle cell anaemia that the 'normal' glycine value did not fall to the adult range[10]. Perhaps the anomaly reported here is responsible for those adult cases of sickle cell anaemia in which, unexpectedly, the glycine value falls in the newborn range rather than in the adult range[11,12]. Whether or not the recently reported $^{T}\gamma$ chain[13] is involved remains to be determined. These problems are under investigation.

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Evidence for localisation of genes for human α-globin on the long arm of chromosome 4

There is disagreement about the site of the human haemoglobin structural genes, mapping of which has been attempted by *in situ* hybridisation[1–4] and family studies[5,6] After *in situ* hybridisation of human metaphase chromosomes with radioactive globin mRNA Price *et al.*[1] suggested that the haemoglobin structural genes are located on the long arm of chromosome 2 and on the long arm of a B-group chromosome, the latter carrying the genes for β, γ and δ chains. Their approach was criticised because of the low specific activity of the ^{3}H-mRNA assay used[7,8], but Atwood *et al.*[2] and Price and Hirschhorn[3,4] confirmed the original assignments using human complementary DNA (cDNA). Finally it was decided that the B-group chromosome that carries haemoglobin loci is chromosome 4[3,4]. Family studies gave results consistent with loose linkage between the locus of the MN blood group and β-thalassaemia. The MN blood group has been mapped tentatively on the long arm of chromosome 2 or on the distal end of the long arm of chromosome 4 (refs 9, 10). But the localisation of the MN system on 2q is inconsistent with data from family studies[11–13], and the distal segment of 4q has been considered more probable[11]. For similar reasons the β-globin locus has been mapped tentatively on chromosome 4 (ref. 5). We report here a study of globin synthesis which confirms that chromosome 4 is the site of the structural gene for α-globin.

We used reticulocytes from patients with congenital aberrations of chromosome 4, which had been demonstrated by R, T, and BUdR-acridine orange banding[14,15]. Case 1 (ref. 16) had duplication 4q28→ 4qter on the long arm of chromosome 2, associated with monosomy 2qter. The chromosomal formula was 46, XY, der (2), dup 4q, t(2;4) (q37; q28). In the healthy mother the same chromosome unbalance was detected in 10% of cells. Case 2 (ref. 16) showed duplication 4q28 → 4qter resulting from maternal translocation t(4; 18) (q28; q22), and was associated with monosomy 18qter. The chromosome designation was 48, XY, der(18), dup 4q, t(4;18) (q28;q22). Case 3 (ref. 16) displayed a "mirror" duplication of chromosome 4 from band q25 to band q34. The designation was 46, XX, der (4), 4qter → 4q 34: : 4q 34 → 4q25: : 4q 34 → qter. Case 4 (ref. 17) was carrier of duplication 4p13 → 4p16, associated with monosomy for the iuxta telomeric region of the long arm of chromosome 4. The designation was 46, XX, rec (4) dup p, inv(4)(p13;q35). The aberrant chromosome was interpreted as an aneusomic recombinant from the father who was heterozygous for a pericentric inversion of chromosome 4.

Red blood cells were drawn, washed and incubated for 1 h with S/^{3}Hleucine, as described by Clegg *et al.*[19] and modified by Riede[20]. Radioactivity and absorbance determinations and specific activity was calculated as described before[18].

As Table 1 shows, results differing significantly from control values were obtained with cases 1, 2 and 3. Two globin synthesis determinations (cases 1 and 3) were also carried out by Dr A. Fantoni (CNEN, Rome), who obtained ratios of synthesis α and β of 1.4 for case 1 and 0.79 for case 3.

Since red cell morphology and haemoglobin A$_2$ levels of the four patients, and their parents were normal, we concluded that cases 1, 2 and 3 had unbalanced globin synthesis of non-thalassaemic origin (pseudo-β-thalassaemia in case 1 and 2, and pseudo-α-thalassaemia in case 3).

Human α and β globins are normally synthesised in equal quantities. But duplication of a chromosome fragment including one of the two globin genes could result in increased production of the globin encoded by the duplicated fragment, with consequent unbalanced globin synthesis.

In cases 1 and 2 part of the long arm of chromosome 4 (bands 28 to 35 included) was duplicated; in both cases an excess of α-globin synthesis was observed. In case 1 the ratio of α/β synthesis was 1.45, which was very close to expectation in erythroid cells with three doses of functional α genes instead of two. Therefore it seemed reasonable to postulate for the diploid cells of this patient the presence of two genes for β globin and, due to chromosome duplication, one additional dose of genes for α globin. Case 2, although morphologically identical to case 1, showed an excess of α-globin synthesis smaller than expected for functional trisomy of the α-globin genetic region. It is possible that in this case the translocated fragment was somehow less active in globin synthesis than normal. Anyway, both cases suggest the localisation of the genes for α-globin on the long arm of chromosome 4 (from band 28 to 35). Band q35 is excluded by the data obtained for case 4 in which this region was deleted and the ratio of α/β globin synthesis was 1.0.

The chromosome abnormality of case 3 was the duplication and inversion of the long arm of chromosome 4 from band 25 to 34, including the same region duplicated in cases 1 and 2. In case 3, however, the ratio of synthesis of α and β globins was significantly below unity.

Observations of case 3 (personal communication from B. Dutrillaux) have shown that in 50–60% of lymphocytes cultivated in the presence of BUdR the long arm of the rearranged chromosome 4 is uncoiled and less fluorescent after acridine-orange staining This suggests the genetic inactivation of the long arm of the chromosome. The suggestion is derived from studies correlating BUdR uncoiling and genetic inactivation in translocations involving the X chromosome[22–27]. In our case despiralisation could involve the segment carrying the α genes, resulting in their inactivation. The erythroid cells of the subject could be therefore a mosaic of two populations, one with three and the other (that with despiralisation) with one

Table 1 Synthesis of α and β globin in reticulocytes from the patients

Case	Chromosome unbalance	Radioactivity (c.p.m.)		Absorbance at 280 nm		Specific activity (c.p.m.)		Ratio of specific activities
		α	β	α	β	α	β	
1*	dup 4q28→4q35	19,450	18,866	18.6	25.9	1,045.6	728.4	1.43
								1.45†
2	dup 4q28→4q35	8,100	7,200	14.0	15.6	578.5	461.5	1.25
3*	dup inv (4)q25→4q34	9,900	14,508	17.1	22.5	577.5	643.6	0.89
								0.79†
4	dup 4p13→4p 16, del 4q35	8,820	9,360	14.55	15.39	606.1	608.1	1.00
Control cases								1.0±0.1

*Data obtained after rechromatography of the α- and β-globin regions.
†Data obtained in a different laboratory, by a different technique.

dose of functional α-globin gene. This would explain the slight defect of α-globin synthesis which we found. The cell population with α-gene inactivation should have a pronounced defect of α-globin synthesis (relative to β-globin synthesis) with consequent accumulation of β_4 tetramers (HbH).

A proportion of the red cells of this subject should therefore contain inclusion bodies. It has not yet been possible to demonstrate such inclusion bodies.

Duplication of the α-globin structural genes has been shown in man[27]. It seems reasonable that the type of duplication fixed by evolution has allowed a balance between α and not α-globin chains; on the contrary this balance has not been respected in the chromosomal aberrations we have studied. On the other hand there is no evidence of chromosomal rearrangements deactivating the regulatory mechanisms of synthesis of structural genes, as observed in patients affected by Down's syndrome (Conconi and Del Senno, unpublished observations).

On the contrary there is considerable evidence for a dosage effect in the case of aneuploidy.

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Vitamin A receptors in normal and dystrophic human retina

Vitamin A is necessary for normal development and functioning of most tissues of the body[1,2]. It is uniquely important for the retina in that, in addition to its possible general role in cellular metabolism, it participates in the visual process as the prosthetic unit for rhodopsin. This takes place in specialised regions of the photoreceptor cell called rod outer segments (ROS). Little is known, however, about uptake, binding and translocation of retinol, the alcohol form of vitamin A, in the photoreceptor unit. A specific 2S receptor for retinol of molecular weight about 17,000 has been described in several animal tissues[3,4]. Retinol receptors are also present in retina and pigment epithelium[5–9]. In addition, we have recently found that an 8S receptor is present in retina and brain of most but not all animal species tested[6]. We report here that normal human retina exhibits both a 2S and 8S binding species for retinol but that only the 2S species is observed in the retina of a patient who had retinitis pigmentosa (RP).

Eyes from two patients with normal retinae and from a patient with retinitis pigmentosa were maintained at 4 °C and used within 4 h of death. The two patients with normal retinae had died of heart disease (48 yr old) and acute lymphocytic leukaemia (17-yr old). The patient with retinitis pigmentosa died from bronchogenic carcinoma at 59 yr. He had extensive loss of peripheral vision, a characteristic finding in the disease. The retinitis pigmentosa in his family is consistent with either the X-linked intermediate or the autosomal dominant type. For histological and electron microscopic examination, a portion of the retinal tissue was fixed in 4% glutaraldehyde in 0.15 M phosphate buffer (pH 7.2) and sections were examined with a JEM-100B electron microscope. For biochemical analysis, retina and pigment epithelium–choroid layers were dissected individually, homogenised in 10 mM Tris buffer (*p*H 7.6, containing 1 mM EDTA, 10 mM KCl and 1mM dithiothreitol) and the 110,000*g* supernatant fraction (cytosol) was prepared[5]. Portions (0.25 ml) of the cytosol were incubated with 1×10^{-7} M ^{3}H-retinol (2.66 Ci mmol^{-1}, New England Nuclear) for 2 h at 4 °C. All operations involving vitamin A were performed in darkness or under dim red light. Samples were layered on 4.6 ml linear sucrose gradients (5–20% sucrose in the Tris buffer) and centrifuged 16 h at 243,000*g*. Approximately 35 fractions were collected per gradient and radioactivity determined as previously described[5]. Protein was measured by the method of Lowry *et al.*[10]. Concentrations were 6.1 and 5.0 mg protein ml^{-1} for normal and RP retinal cytosol samples and 5.6 and 2.0 mg protein ml^{-1} for normal and RP pigment epithelium–choroid cytosol.

Morphologically, the RP retina and pigment epithelium exhibited degenerative changes with marked loss of photoreceptor cells. Appreciable numbers of cone cells remained in the macula, but few of these had outer segments (Fig. 1*a*). Outer segments were detected only in the foveal area and were considerably disorganised as assessed by electron microscopy (Fig. 1*b*). Thus the sample was typical of advanced retinitis pigmentosa.

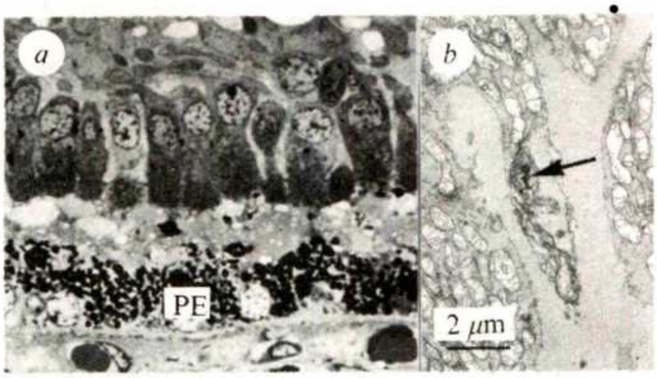

Fig. 1 *a*, Photoreceptor cells and pigment epithelial cells in the parafoveal area (× 660). Although the outer segments are generally missing in the area, cone cell bodies (C) are still present. The pigment epithelial cells (PE) contain abundant lipofuscin granules. *b*, Disorganised lamellar membranes within the outer segment of a cone cell are still recognisable (arrow) in the foveal area (× 4,320).

As assessed by sucrose density gradient centrifugation, two binding species for ^{3}H-retinol were observed in the cytosol fraction of normal human retina (Fig. 2*a*). Sedimentation coefficients are approximately 2S (peak at fraction 26) and 8S (peak at fraction 13). Under the present conditions, 180 and 900 fmol of vitamin A were bound per mg of total protein in the 2S and 8S peaks respectively. In the cytosol fraction of the RP retina, the 8S peak was completely absent. The 2S peak was comparable with that seen in the normal retina with 240 fmol of vitamin A bound per mg protein.

With normal pigment epithelium–choroid cytosol, a small 8S peak was seen along with a larger 2S peak (Fig. 2*b*). A 2S peak was observed in the cytosol fraction from the RP eye but the 8S peak seemed to be absent. Since the protein concentration was lower in the RP cytosol sample, binding in the 2S region of RP pigment epithelium–choroid cytosol was actually somewhat higher than in the normal case with 590 fmol and 800 fmol vitamin A bound per mg protein for normal and RP cytosol samples respectively. The 8S peak observed in the pigment epithelium–choroid preparation may be endogenous to the pigment epithelium or alternatively could be ascribed to ROS contamination. Even using the most favourable conditions, when normal retina is separated from pigment epithelium, a variable percentage of photoreceptor tips breaks off and remains entrapped in the microvilii of the pigment epithelium. In the RP eye, the lack of this small 8S peak may then be correlated with the virtual absence of ROS in the retina and gives supportive evidence for compartmentalisation or concentration of the 8S receptor species in ROS. Attempts to identify retinol receptors in purified ROS preparations have so far been unsuccessful, most probably because the receptors are soluble and are easily lost from ROS membranes during purification procedures.

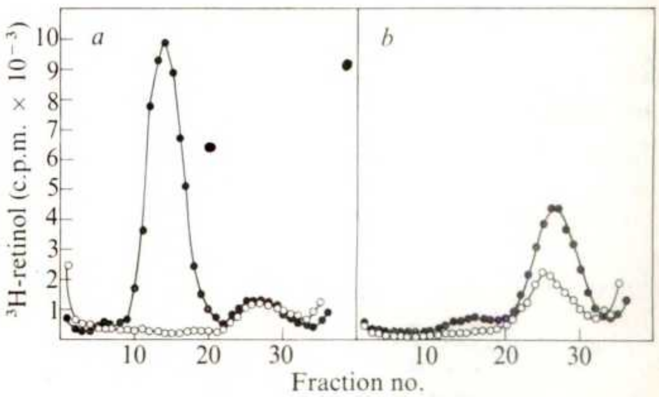

Fig. 2 *a*, Sucrose density gradient centrifugation patterns of human retinal cytosol fraction incubated with 0.1 μM ^{3}H-retinol. ●, Normal retina; ○, RP retina. *b*, Patterns with human pigment epithelium–choroid cytosol fraction incubated with 0.1 μM ^{3}H-retinol. ●, Normal pigment epithelium–choroid; ○, RP pigment epithelium–choroid.

These data indicate that not only does the human retina contain two distinct receptor species for retinol, but that these may be compartmentalised in different retinal areas. If one assumes that the 8S species is solely associated with outer segments, one would then expect an absence of this receptor species in the severely affected RP retina examined. In this way the RP retina serves as a model for a 'rodless' retina. Sequestering of the 8S species in an organelle as specialised as the photoreceptor unit would strongly implicate the receptor in the visual cycle, possibly as a transport vehicle for vitamin A between retina and pigment epithelium.

Lack of the 8S binding species may also be correlated with a specific biochemical abnormality resulting in or genetically linked to retinitis pigmentosa. Study of the receptors both qualitatively and quantitatively in retinae from young patients if they become available for research will clarify this point. In any event, the presence of at least two receptor species for retinol in normal human retina and the possible compartmentalisation of the 8S species in the photoreceptor unit suggest an important role for such receptors in normal retina functioning.

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Identification of a membrane protein binding the retinol in retinal pigment epithelium

AVAILABLE experimental evidence indicates the primary role of retinal pigment epithelium (PE) in the uptake of retinol from the extravascular space of the choriocapillaris[1] and possibly in its transfer to and from the photoreceptors[2]. While the transport mechanism of retinol in plasma by the retinol-binding protein (RBP) is known in detail[3,4], only partial information is available on how circulating retinol is transferred from its carrier protein to PE. It has been shown[5] recently that iodinated human RBP binds specifically to isolated bovine PE and autoradiographic investigations[6] have suggested that the binding is confined to the choroidal surface of these cells. We have previously demonstrated[7] that isolated human and bovine PE actively binds ^{3}H-retinol only if this is present in the incubation medium as a complex with human RBP, and suggested that the interaction of circulating RBP with the cell membranes of PE is specific (G.M. and F.G., unpublished observations), and essential, for the active transport of retinol in these cells against a concentration gradient. We have now identified, in the plasma membranes of bovine PE incubated in the presence of ^{3}H-retinol-RBP, a binding protein for retinol; some evidence is presented which suggests that this membrane protein is different from plasma RBP.

The RBP used in these experiments was prepared from human plasma as described by Peterson and Berggard[4] and purified by affinity chromatography on prealbumin-coupled Sepharose 4B according to Vahlquist *et al.*[8] Retinol was then extracted from the lyophilised RBP with ethanol and ^{3}H-retinol (specific activity 2.4 Ci mmol^{-1}) substituted for the original chromophore in the apo-RBP following the procedure described in detail by Heller and Horwitz[9]. The reconstituted ^{3}H-retinol-RBP was again purified on the same affinity chromatography column in order to eliminate excess ^{3}H-retinol and possible residual apo-RBP by utilising the inability of retinol-free RBP to bind prealbumin[8,10].

PE was prepared from bovine eyes immediately after death as previously described[7]. Such PE preparations show no appreciable contamination by rod outer segments nor by choroidal remnants, and they actively synthesise proteins in short term (2 h) incubation experiments[7]. After the last washing the PE pellet was carefully resuspended in 2 ml of the same medium containing ^{3}H-retinol-RBP (0.1 nmol ml^{-1}) and incubated for 1 h at 37 °C in a water bath with occasional gentle stirring. At the end of the incubation period the cells were separated by centrifuging, washed twice in ice-cold unlabelled medium, homogenised in 1 ml of medium with a tight-fitting Teflon glass homogeniser and separated on a discontinuous sucrose gradient. By this method it was possible, as shown by electron microscopy of fixed samples of the different bands collected at the gradient interfaces, to separate the plasma membranes of PE clearly from the soluble cell sap and from the other cellular organelles. The gradient was then scanned at 280 nm and tested for radioactivity. The gradient pattern and the distribution of radioactivity of a typical experiment are reproduced in Fig. 1. Inspection of the figure shows clearly that radioactivity is practically limited to the two most superficial gradient bands, that is, those corresponding to the soluble cell sap and to the plasma membranes of PE. Although the ratio between turbidity and radioactivity is substantially similar for the two peaks, as much as 75% of the radioactivity is found in the plasma membrane fraction.

The fractions corresponding to this main peak were pooled and diluted with Krebs Ringer solution and the plasma membranes were collected by centrifuging at 35,000 r.p.m. in a SW40 rotor for 2 h. This washing procedure did not cause the removal of significant amounts of the membrane-bound retinol since only about 6% of the original radioactivity was found in the resulting supernatant fluid. The plasma membrane pellet was then dissolved in a detergent solution and analysed for protein and radioactivity distribution by polyacrylamide SDS electrophoresis (7.5–12% acrylamide). The gels were calibrated for molecular weight determination using serum albumin (molecular weight 40,000), soybean trypsin inhibitor (molecular weight 20,000), human RBP (molecular weight 21,000), myoglobin (molecular weight 17,000) and cytochrome c (molecular weight 11,700).

Of the different detergent solutions used to solubilise the membrane preparations, 2.5% sodium dodecyl sulphate (SDS) was found to give the best recovery in the gels (over 90% of the radioactivity originally present in the sample). In more damaging conditions (4% SDS) this recovery was only 5%.

The gel staining pattern and the distribution of radioactivity indicate that all the ^{3}H-retinol present in the gel is bound to a single Coomassie-blue stained band. Typical results are reproduced in Fig. 2. The electrophoretic mobility of this protein band is higher than that of native human RBP and indicates a molecular weight of approximately 14,500. This band follows in the gel a minor Coomassie-blue staining band which has a molecular weight

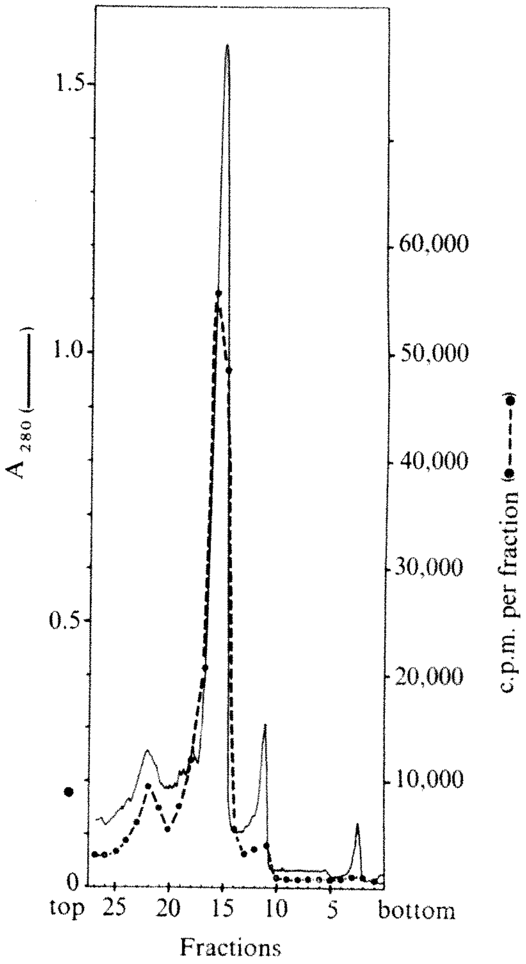

Fig. 1 Scanning and labelling profiles of homogenate of bovine PE preincubated with ^{2}H-retino-RBP and fractionated on a discontinuous sucrose gradient by centrifuging for 150 min at 27,000 r.p.m. in a SW 40 rotor with a L_2 Spinco ultracentrifuge. The following sucrose solutions were layered in the centrifuge tube from bottom to top: 44%, 40%, 36%, 33%, 15%. The gradient was scanned at 280 nm utilising a LKB pump and a Pye-Unicam SP 1700 spectrophotometer. Fractions of 0.5 ml were collected. 15μl aliquots of each fraction were tested for radioactivity in a Tricarb scintillation spectrometer (model 2425).

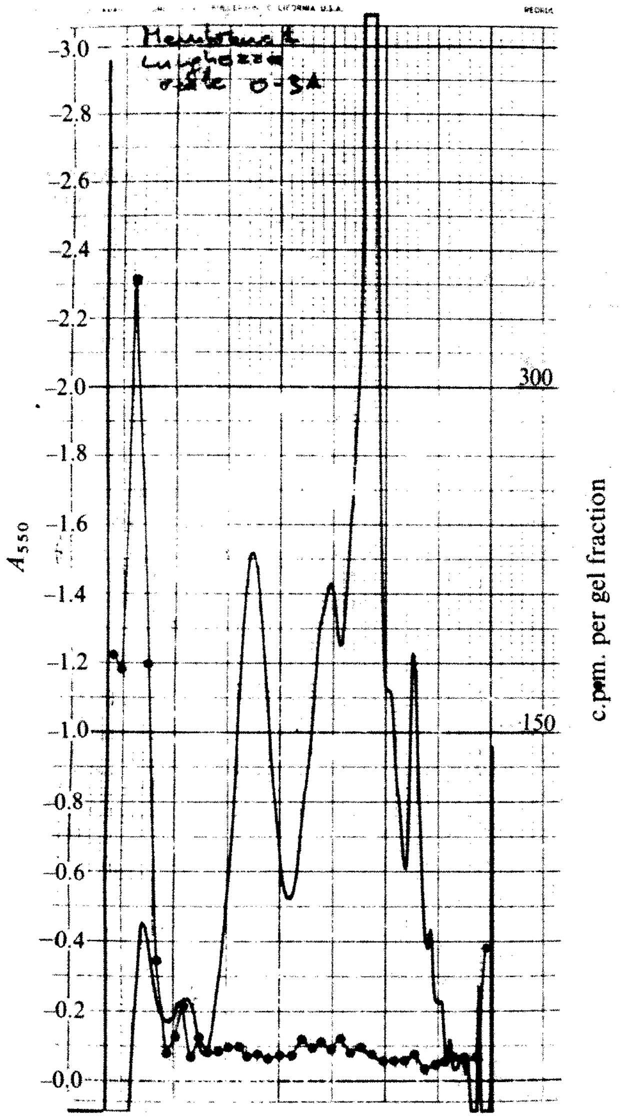

Fig. 2 Staining and labelling profiles of plasma membrane proteins from bovine PE preincubated with ^{3}H-retinol-RBP. Plasma membranes were dissolved overnight at room temperature with 2.5% SDS and 1% dithiothreitol in 0.3 M Tris-Cl$^-$ buffer (*p*H 8.9) and analysed for protein and radioactivity distribution by polyacrylamide SDS gel electrophoresis. The gels contained 7.5% acrylamide (stock solution 30% acrylamide, 0.8% *n-n'*-methylene-*bis*-acrylamide). The amount of protein applied to each gel was 50 µg. Gels were fixed and stained with Coomassie-blue. Scanning of the gels was performed at 550 nm. Duplicate unstained gel was sliced into 2.3 mm fractions, dissolved in H_2O_2 at 45 °C for 2 d, and resuspended in Toluene 70%, Omnifluor 0.4% metylcellosolve 30%. Radioactivity was determined by using a Tricarb scintillation spectrometer, with a counting error less than 1%.

of approximately 20,000 but which does not appear to contain appreciable radioactivity.

From the stained gel, after scanning, the protein band containing radioactivity was cut off. The gel slice was extracted three times with methanol and chloroform. The pool of the extracts was concentrated, applied on a thin layer of silica-gel F 254 and developed in the dark with a mixture of cyclohexane–ethanol 97/3 (ref. 11). Under ultraviolet light the chromatogram for reference retinol presented a principal spot with $R_f=0.51$ and two minor spots with $R_f=0$ and $R_f=0.95$ (oxidation products, probably the acid and the aldehyde). The sample zones corresponding to the three reference spots were scraped off, put in vials using 4% (w/v) Cab-O-Sil as gelling agent, and counted. Pure silica gel from the plate was scraped off as a blank. Radioactivity was present only in the principal spot ($R_f=0.51$); recovery of the gel radioactivity was 18%.

In four experiments isolated PE was incubated in the presence of iodinated human plasma RBP. Iodination of native RBP with ^{125}I sodium iodine and chloramine-T was performed as described by Heller[5]. After incubation with ^{125}I-RBP PE cells were treated as previously described. In none of the experiments could appreciable radioactivity be detected in the plasma membrane pellet at the end of the isolation procedure.

The gel staining pattern of plasma membranes of native PE (that is, not preincubated in the presence of RBP) is identical to that reproduced in Fig. 2 indicating that, under the experimental conditions used, no detectable change in the membrane protein composition was caused by the incubation with RBP.

Present results indicate that a protein able to bind retinol maybe extracted from the plasma membranes of PE incubated in the presence of exogenous RBP and is probably involved in the process of active retinol uptake by these cells. The molecular weight of this protein, calculated by SDS gel electrophoresis according to Weber and Osborn[12], is of the order of 14,500. This observation together with our inability to detect appreciable amounts of ^{125}I-RBP in the membrane preparation at the end of the isolation procedure suggests that the retinol-binding protein extracted from PE membranes is not the exogenous RBP present in the incubation solution. The same protein band was identified in SDS extracts of native PE membranes, which suggests that this protein is a normal constituent of this membrane. Nothing can be said at present about the possible relationship of this membrane protein with the low molecular weight retinol-binding proteins (molecular weight 17,000) recently demonstrated[13] in the cytosol fraction of PE.

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Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells

OESTROGENS are more readily accumulated and retained in responsive cells than in cells that are not their targets[1]. Cytoplasmic macromolecules[2] which specifically interact with oestradiol and other steroid hormones seem to mediate transfer of the agonist to the nuclear chromatin, where the complex is believed to promote expression of the phenotypic effects[3–6]. It is generally assumed that the hormone diffuses passively to "cytoplasmic" receptors which determine the cellular specificity of response[7]. But some experiments indicate that steroid hormones interact with components of biological membranes and may enter their respective target cells by a membrane-mediated process[8-12] which is saturable and temperature-dependent[13-17]. We have investigated steroid-binding components associated with the plasma membranes of cells isolated from endometrium, liver and intestinal mucosa. Endometrial and liver cells show substantial binding to oestrogen immobilised by covalent linkage to an inert support, while intestinal cells have no such binding sites. The relative quantity of these steroid receptors at the outer surfaces of cells from diverse tissues corresponds well with the capacity of a given cell to accumulate and retain oestrogen.

Uterus, liver and intestine were excised from female rats that had been ovariectomised at 6 weeks old and maintained for 3 weeks in a low-steroid environment[16]. Epithelial cells were isolated from the mucosal surfaces of uterus and intestine by methods described before[18]. In brief, the procedures involved incubation with 20 mg of collagenase (Type I, Worthington) per 100 ml of Ca^{2+}-, Mg^{2+}-free Ringer, followed by restoration of divalent cations. Complete Ringer solution consisted of 136.9 mM NaCl, 2.7 mM KCl, 0.6 mM $CaCl_2$, 0.5 mM $MgCl_2$, buffered at *p*H 7.4 with 8.1 mM Na_2HPO_4 : 1.5 mM KH_2PO_4. Liver cells were isolated from excised tissues essentially by the method of Howard and Pesch[19] with 200 mg of collagenase per 100 ml of the above media. Cellular debris and damaged cells were removed from the cell suspensions as described before[12,18]. As a restorative procedure, the cells were incubated for 120 min in complete Ringer solution enriched with 0.1% (w/v) highly purified albumin (Pentex) and 1 mM sodium pyruvate before the start of experiments. Throughout these and all subsequent manipulations, at least 90% of all cells excluded the vital dye, Trypan blue, at a final concentration of 0.05% in Ringer solution during a 10-min incubation at 22 °C.

For determinations of total cellular uptake of free oestradiol, cells were diluted with fresh Ringer solution to a final concentration of 1×10^7 cells per ml. Incubations (5–60 min) were begun with the addition of 6, 7-^{3}H-oestradiol-17β (99 Ci mmol^{-1}, New England Nuclear; final concentration, 1×10^{-9} M) at 22 °C in oxygen. A 200-fold excess of unlabelled oestradiol-17β ($E_2\beta$) was added to paired samples for determination of displaceable or nonspecific binding[14]. Incubations were terminated by dilution with 4 volumes of ice-cold Ringer solution. The cells were then sedimented by centrifugation at 800 *g* (4 °C) for 5 min. The resulting cell pellets were washed three times by resuspending them in ice-cold Ringer solution and centrifuging as before. The washed pellets were sonicated at 0–4 °C, and the radioactivity in samples of the disrupted cells was determined by liquid scintillation spectrophotometry with 40–43% efficiency. Additional aliquots of the sonicate were analysed by the method of Hill and Whatley[20] for DNA.

To prepare oestrogen-derivatised supports for determinations of cell binding, fibres of transparent nylon monofilament (size 50, Penn Products), were strung into polyethylene rings (cut from S-6 stoppers, Mallinckrodt) as described by Edelman *et al.*[21]. The mounted fibres (8 mg nylon per ring), in individual Petri dishes (10×35 mm), were extracted to remove surface contaminants for successive 10-min periods with petroleum ether, carbon tetrachloride, 80% (v/v) methanol and water. The surface of the nylon was then partially hydrolysed with 3 N HCl for 30 min at 30 °C[22]. After thorough washing with water to arrest further hydrolysis, the fibres were activated with 12.5% (v/v) glutaraldehyde (grade I, Sigma) in 0.1 M phosphate buffer, *p*H 7.4, at 37 °C for 24 h (ref. 23). The reaction was terminated by exhaustive washing of the fibres with the buffer. The activated fibres were next in phosphate buffer and incubated for 18 h at 4 °C. Fibres placed in Petri dishes containing 5 ml of 2% (w/v) albumin were then washed with buffer to remove albumin that was not covalently bound to the activated nylon[23]. Samples of the combined washes were taken to determine protein by the method of Lowry *et al.*[24]. The introduction of albumin on the fibre was 0.12 mg per mg of nylon.

Oestradiol hemisuccinate was coupled to the albumin : nylon fibre with carbodiimide by procedures similar to those described by Sica *et al.*[25]. The protein-derivatised fibres were transferred to 20-ml beakers in which were placed 10 ml of 70% (v/v) aqueous dioxane containing 17β-oestradiol 17-hemisuccinate (272 μg per mg of albumin, Sigma). Two 50-mg portions of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Aldrich) were added (at 0 and 4 h). The samples were rocked gently for 18 h at 22 °C. The fibres were then washed at the same temperature successively with a total volume of 500 ml of dioxane (1 h), 5 l of 80% methanol (72 h), and 500 ml of water (15 min). The oestrogen-derivatised fibres were transferred to fresh Petri dishes, dried and stored *in vacuo* at 0–4 °C until use. The amount of covalently bound oestrogen was determined in three samples by incubation of derivatised fibres (0.36 mg albumin) with 2 ml of 0.5 M NaOH at 22 °C for 2 h. After stoichiometric neutralisation with HCl, the free oestradiol was

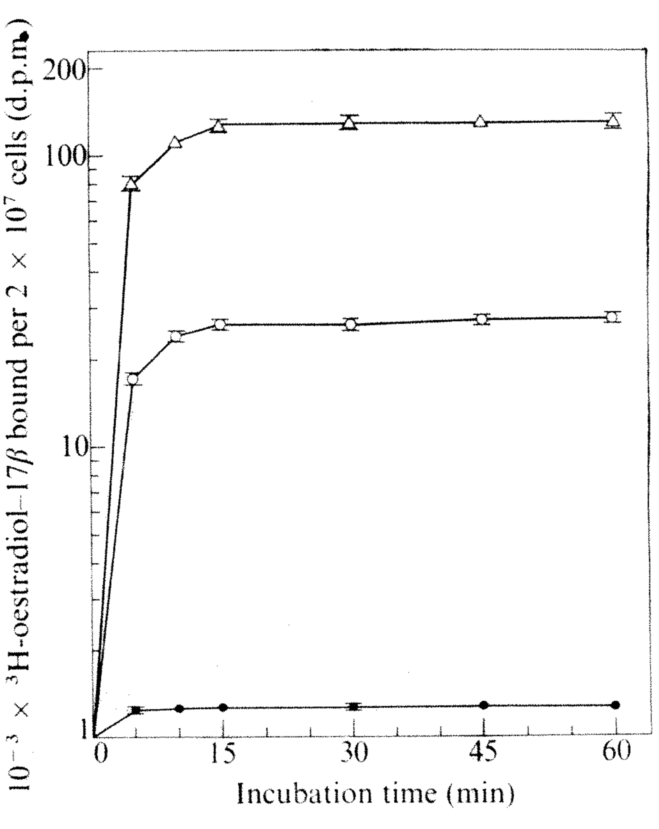

Fig. 1 Uptake of ^{3}H-oestradiol-17β by intact cells. Cells isolated from intestinal mucosa (●), liver (○) and endometrium (△) were incubated at 22 °C for 5–60 min with 1×10^{-9} M ^{3}H-$E_2\beta$ as described in the text. Only specific binding (defined as the difference in bound steroid between paired tubes, one of which contained a 200-fold excess of unlabelled $E_2\beta$ throughout the experiment) is shown. Specific binding of $E_2\beta$ by liver and endometrial cells (22 °C, 30 min) was not reduced significantly in the presence of the relatively inert oestradiol-17α at a concentration of 2×10^{-9} M ($P > 0.80$). Among liver and endometrial cells incubated at 4 °C for 30 min with 1×10^{-9} M ^{3}H-$E_2\beta$, specific oestrogen binding was reduced to 26% of that bound to paired cells maintained at 22 °C (both at $P < 0.01$, Student's *t* test). The extent of $E_2\beta$ binding is expressed relative to cell numbers, as derived from determinations of cellular DNA content (for example, 8.3, 10.6 and 9.3 μg of DNA per 10^6 cells of endometrium, liver and intestinal epithelium, respectively). Detailed analyses of specific oestrogen uptake, to be presented elsewhere, indicate that binding sites per endometrial, liver and intestinal mucosal cell average ~ 24,000, 3,400 and 50, respectively.

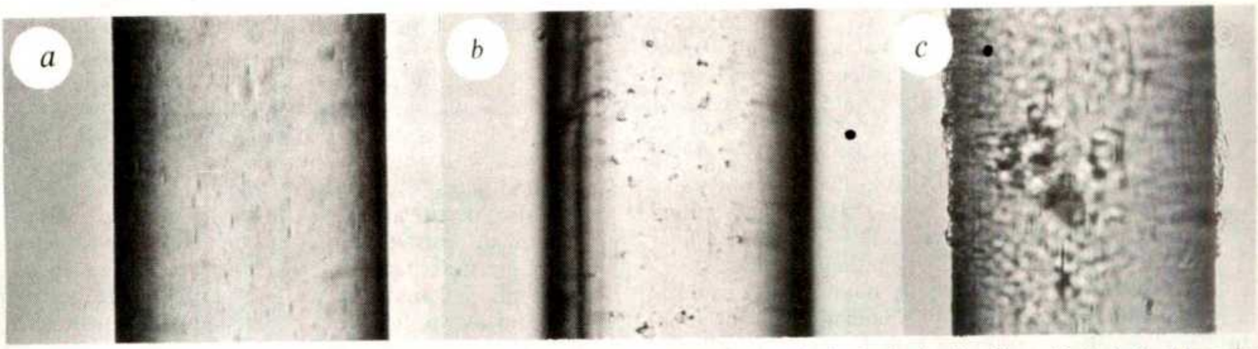

Fig. 2 Binding of isolated cells to oestrogen immobilised by covalent linkage to albumin-derivatised nylon fibres. Cells derived from intestinal mucosa (*a*), liver (*b*) and endometrium (*c*) were incubated with the derivatised fibres by addition of 1×10^7 cells per 4 ml of Ca^{2+}-, Mg^{2+}-free Ringer solution to a 10×35-mm Petri dish with the mounted fibres (8 mg of nylon per dish). The dishes were placed on a platform shaker (80 oscillations per min) at 22 °C for 30 min, after which unbound cells were separated by complete immersion of the dish successively in a series of three beakers, each containing 10 volumes of the incubation medium. The washed fibres with retained cells were then transferred to 4 ml of fresh incubation medium for photomicrography, using an immersion lens and Kodak Tri-X Panatomic film (ASA 400). Significant numbers of hormone-responsive cells (that is endometrial and liver cells) adhere to the fibres, whereas "nontarget" cells from intestine are poorly retained. No binding of any cells was observed when underivatised fibres or fibres coupled only to albumin were used (×250).

extracted with four equal volumes of diethyl ether (Mallinckrodt) that had been freshly saturated with double distilled water. The combined ether extracts were repeatedly backwashed with water, and evaporated at room temperature with a stream of nitrogen. Oestrogen was determined in the resultant residue by radioimmunoassay[26] (courtesy of Dr R. Swerdloff). The minimal substitution of oestradiol was 1 ng per mg of albumin.

Figure 1 shows the extent of uptake of 1×10^{-9} M ^3H-$E_2\beta$ by intact cells isolated from endometrium, liver and intestinal mucosa. The specific accumulation reported here represents the amount of radioactivity not displaced by the addition of a 200-fold excess of unlabelled $E_2\beta$. Among all cells incubated at 22 °C, accumulation of $E_2\beta$ was essentially maximal within 10–15 min, and there was no further increment in uptake of steroid for up to 60 min of incubation. As anticipated, endometrial cells retained the most $E_2\beta$ per cell. The moderate level of hormone uptake observed in liver cells is consistent with evidence for the presence of specific oestrogen receptors in rat liver[27,28]. The least retention was seen with epithelial cells from intestine, which is considered not to be a target tissue for oestrogen[29]. In addition, $E_2\beta$ binding to liver and endometrial cells maintained at 4 °C for 30 min was approximately four times less than that bound to corresponding cells incubated in the same conditions, but at 22 °C (Fig. 1).

To probe for steroid-binding sites at the external surfaces of cells derived from endometrium, liver and intestinal mucosa, isolated cells were incubated with the mounted 17β-oestradiol-17-hemisuccinyl: albumin: nylon fibres in the standardised conditions indicated in Fig. 2. Cells retained by the oestrogen-derivatised fibres after extensive washing were observed by microscopy. Significant numbers of hormone-responsive endometrial and liver cells were found to adhere to the steroid-derivatised fibres, but nontarget cells from intestine were essentially not retained (Fig. 2). Since there was no association of cells when unmodified nylon fibres or fibres derivatised only with albumin were used, the binding to oestrogen-derivatised fibres by target cells exclusively must be attributable to the presence of the steroid and the occurrence of specific recognition sites at the external surfaces of given cells.

A quantitative estimate of cell binding to the derivatised fibres was obtained as described in Fig. 3. Most fibre-bound cells could be displaced and recovered free and apparently intact by competitive inhibition of binding with excess $E_2\beta$, but not with oestradiol-17α($E_2\alpha$) added to the medium. Remaining cells were dislodged by brief exposure to hypotonic saline[21]. Among all cell types, the number of bound

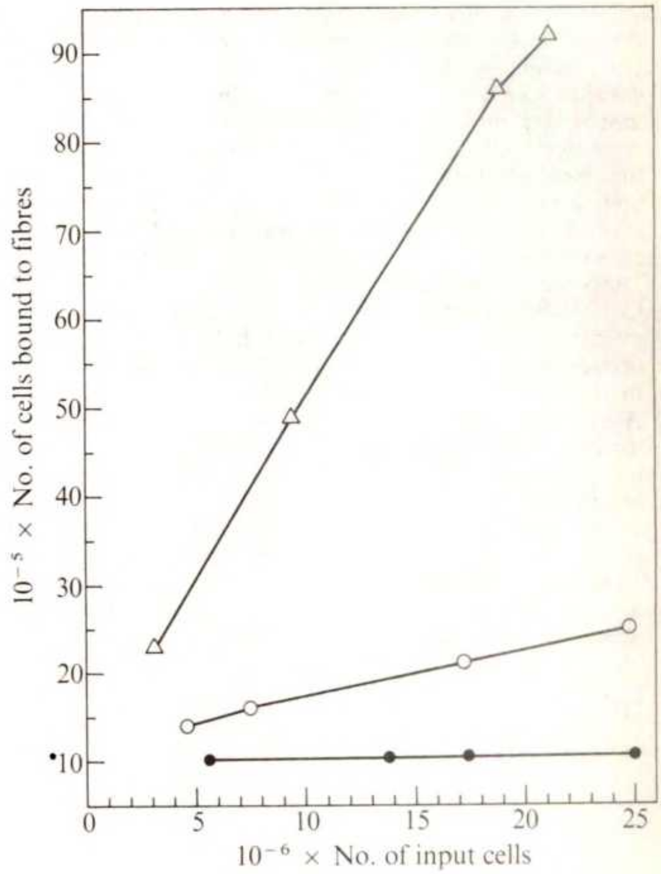

Fig. 3 Extent of cell binding to oestrogen immobilised on nylon fibres. Cells isolated from intestinal mucosa (●), liver (○) and endometrium (△) were incubated with the fibres by the addition of 3–25×10^6 cells per 4 ml of medium using standard conditions of mixing and shaking (Fig. 2). After removal of unbound cells, cells attached to the derivatised fibres were dislodged by incubation for 1 h in divalent cation-free Ringer solution containing 2×10^{-7} M $E_2\beta$, followed by 1 min of incubation in hypotonic (150 mosm) saline[25] with excess $E_2\beta$. ($E_2\beta$. ($E_2\alpha$ was ineffective in displacing cells from their association with fibres.) Cells recovered by these procedures were disrupted by sonication at 4 °C, and samples of the sonicate were taken for determination of cellular DNA contents. Cell numbers reported here were derived from measurements of DNA content per cell (Fig. 1). Each data point represents the mean of two or three independent experiments. The relation of the number of fibre-bound cells to total cells present in the incubation medium is defined by the slope, 0.003 for intestinal cells ($r = 0.98$), 0.054 for liver cells ($r = 0.99$), and 0.386 for endometrial cells ($r = 0.99$).

cells was a linear function of the cell concentration over a wide range (Fig. 3). This result suggests that, in the conditions specified, binding is proportional to the number of input cells; the binding of one cell is independent of that of others, and the fibre surface is not saturated with cells. As in analyses of total cellular uptake of $E_2\beta$ (Fig. 1), the capacity of endometrial cells to bind oestrogen coupled to a fibre surface surpassed that of cells from liver and intestine. The percentage of total input cells that become bound averaged 40±1 (9) for endometrial cells, 7±1 (8) for liver cells, and 0.3±0.1 (8) for intestinal epithelial cells.

The number of endometrial cells bound to derivatised fibres was significantly reduced by incubation at low temperature and also by exposure of cells to free $E_2\beta$ followed by three washes at 400*g*, before incubation of the cells with fibres in the standard conditions (Table 1). Binding of oestrogen at the outer surfaces of cells incubated at 4 °C was four times less than that to paired cells maintained at 22 °C. Association of $E_2\beta$-pretreated cells with derivatised fibres (at 22 °C) amounted to 42% of that of corresponding endometrial cells exposed only to hormone vehicle, ethanol, at a final concentration of 0.02% ($P<0.01$). In contrast, prior incubation of these cells with $E_2\alpha$ elicited no significant reduction in the subsequent binding of washed cells to the test fibres compared with that of control cells ($P>0.90$).

These studies provide direct evidence that receptor components specific for oestradiol-17β, but not for the 17α-isomer, are present at the external surfaces of oestrogen-responsive cells. These results seem to be in conflict with the assumption that steroid hormones enter all cells by nonspecific, free diffusion[6,30]. Several investigators, however, have noted marked saturability and temperature-dependence of steroid hormone entry, which cannot be attributed to the function of cytoplasmic receptors[13-17]. Our studies are consistent with these observations. Moreover, the decline in oestrogen accumulation by target cells at reduced temperature may be attributable to a reduction in the availability of surface receptors for the hormone. Alterations in the physical state and orientation of various membrane macromolecules may contribute to the reduced interaction of oestrogen with surface binding components at low temperatures[13,31-33].

The number of surface sites available for binding to oestrogen-derivatised fibres is markedly diminished in endometrial cells after exposure to oestradiol-17β, but not after treatment with the relatively inactive 17α-isomer. This may be attributable to masking of the external sites by bound oestrogen or to a hormone-induced change in the location or orientation of the oestrogen-binding components. Other studies have shown that target-selective uptake of specific antigens is associated with localised disordering of membrane structure and subsequent internalisation, presumably of those regions of the surface membrane equipped with, or immediately adjacent to, specific recognition sites[34-36]. A corresponding sequence of events may also occur at the surfaces of endometrial cells in response to oestrogen, as is evident from reports of altered membrane functions[12,37,38] and micropinocytotic vacuolation of the plasmalemma[38] after brief exposure to oestrogen.

Table 1 Effect of temperature and prior treatment with oestrogens on the binding of isolated endometrial cells to oestrogen immobilised by covalent linkage to albumin-derivatised nylon fibres.

Incubation temperature (°C)	Treatment before incubation	No. of fibre-bound endometrial cells number of input cells (%)
22	None	40±1 (3)
4	None	10±1 (3)*
22	Oestradiol-17α ($1+10^{-8}$M, 1 h)	39±2 (3)
22	Oestradiol-17β ($1+10^{-8}$M, 1 h)	17±2 (3)†

The extent of endometrial cell binding to oestrogen-derivatised fibres at 22 °C or 4 °C was determined as described in Figs 2 and 3. The number of cells added to each incubation vessel, the input cells, averaged 2×10^7 (≃166 μg DNA). In some experiments, cells were treated for 1 h with 1×10^{-8} M $E_2\beta$ or $E_2\alpha$ and then washed three times in steroid-free medium at 400*g* (5 min) before exposure to the derivatised fibres in the standardised conditions. All data are presented as mean±s.e.m. with the number of determinations in parentheses.

* Value significantly different from appropriate control at $P < 0.001$.

† Value significantly different from appropriate control at $P < 0.01$ (Student's *t* test).

In contrast to numerous reports devoted to the identification of membrane-associated receptors for polypeptide hormones[39,40], membrane-binding sites with specificities for steroid hormones have scarcely been investigated. Our work provides direct proof that such sites exist at the surface membrane of cells responsive to oestrogen. But the nature and origin of these binding components and the manner in which they may contribute to the recognition and mediation of entry of oestrogenic hormones remain to be determined.

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Abnormal myelination in transplanted Trembler mouse Schwann cells

AXON and Schwann cell interdependence has received increasing attention in recent years[1,2]. In normal nerves, axons influence Schwann cell proliferation[3,4] and myelin formation[5-8]. These interactions may also be important in peripheral nerve disorders. Indeed, it has been suggested that in certain neuropathies, it is an axonal abnormality which is responsible for the failure of Schwann cells to maintain normal myelin[9,10]. An experimental approach to this question has become possible with the demonstration that, in nerve grafts from normal animals, Schwann cells which originate in donor nerves ensheath and myelinate axons arising from nerve cells in the host[6,8,11]. This demonstration of the feasibility of *in vivo* combinations of Schwann cells and axons, each originating from different animals, prompted the present experiments in which axons and Schwann cells from normal and abnormal mice were combined in sciatic nerve grafts. For the present study, abnormal nerves were obtained from Trembler mice (Tr)[12]. These mutants have a dominantly inherited neuropathy characterised by the presence of abnormally thin myelin sheaths and slow conduction in the peripheral nerves[13], changes which have been considered to resemble those of human hypertrophic neuropathies[14].

To define further the abnormality in Tr neuropathy, sciatic nerves and spinal roots from six affected mice and five control C57BL/6J mice aged 14 d to 7 month were examined by phase and electron microscopy[6,15]. In normal nerves, most axons greater than 1 μm in diameter were surrounded by Schwann cells containing a myelin sheath; in Tr nerves, between 30 and 70% of Schwann cells ensheathing fibres of this size lacked myelin completely and the remainder had thin myelin sheaths (hypomyelination). Complete counts of axons and Schwann cell nuclei were obtained from electron microscope montages of whole transverse sections taken from the mid-portion of the 10 to 15 mm long L4 ventral roots of four adult control and Tr mice[15]. The average number (±s.e.) of axons in controls (914±17) and Tr mice (856±55) was similar. Schwann cell nuclei were of equal size in both groups of animals, but there was a 10-fold increase in the number of Schwann cell nuclei in L4 ventral roots of Tr mice compared with controls (266±24 and 22±4 respectively). Although this hypercellularity in Tr nerves could be due to a chronic demyelinating process[16,17], it may also be secondary to a developmental failure of myelination: the length of Schwann cell territories in teased fibres of Tr mice of all ages studied has been found to be less than normal (unpublished work). Presumably because of the interdependence of myelin thickness and the length of Schwann cell territories[18], both the radial and the longitudinal extension of these cells may be similarly deficient in Tr mice nerves; as a result, axonal ensheathment in fibres which are hypomyelinated or lack myelin must only be possible through a neonatal increase in Schwann cell number. Finally, there were no inflammatory cells or macrophages in Tr sciatic nerves or roots, and neither myelin debris, an indicator of active demyelination, nor concentrically arranged Schwann cell processes (onion bulbs)[16,17] were prominent features in the Tr nerves we have examined.

Because these quantitative and morphological findings indicated Schwann cell abnormalities and no axonal loss in Tr neuropathy, interactions between axons and Schwann cells were investigated in sciatic nerve grafts. In 26 mice, approximately 6 week old, a 5 mm segment of the sciatic nerve was removed from a donor mouse and immediately grafted between the cut ends of recipient sciatic nerves at the mid thigh level. The graft was secured to host nerve

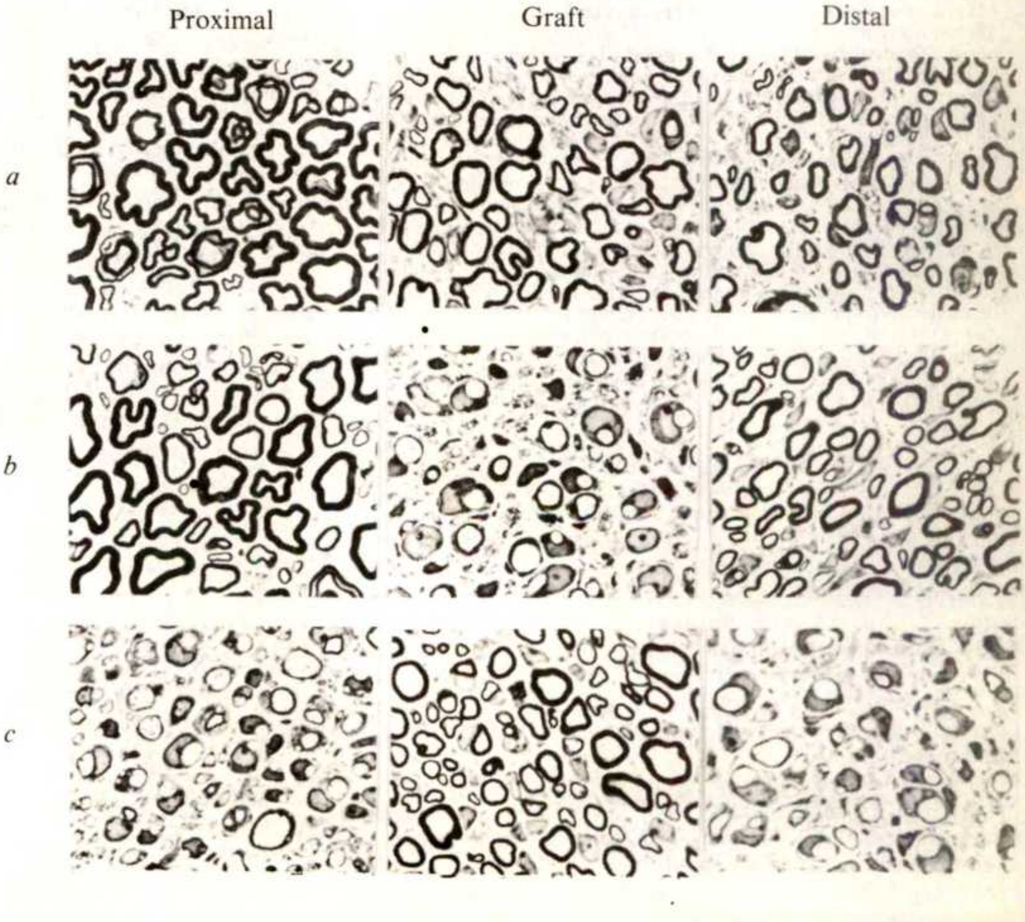

Fig. 1 Phase micrographs of sciatic nerves at three different cross-sectional levels 2 months after grafting. Sections were taken approximately 3 mm from the graft (proximal stump), the mid-portion of the graft, and 3 mm distal to the graft (distal stump). *a*, Normal-to-normal grafts. Grafted segments and distal stumps contain numerous regenerated myelinated fibres which closely resemble those in the proximal stump. *b*, Trembler-to-normal grafts. Proximal and distal stumps (recipient nerves) appear normal, while fibres within the graft either lack myelin or are hypomyelinated. *c*, Normal-to-Trembler grafts. Proximal and distal stumps of host nerves show many fibres with little or no myelin but the morphology of fibres in the graft resembles that of normal regenerated nerves.

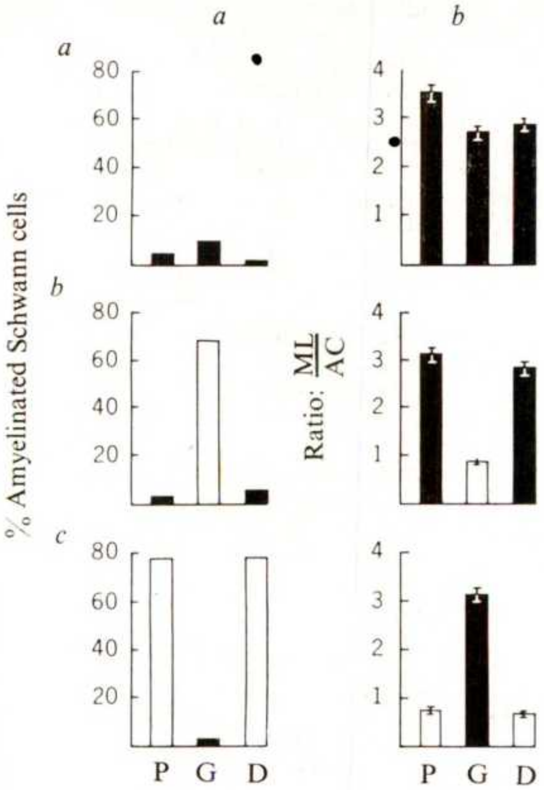

Fig. 2 Proportions of nerve fibres with Schwann cells containing no myelin (*a*) and ratios of the number of myelin lamellae to axonal circumference (ML/AC) in myelinated fibres (*b*) from cross sections of proximal (P), graft (G) and distal (D) segments from sciatic nerves 2 months after grafting. Solid bars represent data from normal nerve segments; open bars symbolise values in segments from Trembler nerves. The percentage of fibres with no myelin, calculated at each level for an average of 230 axons greater than 1 μm in diameter, is expressed as mean ± s.e.m. The ML/AC was measured in 100 fibres at each level from electron micrographs at a final magnification of × 9,100. In N–N–N nerves, fewer than 10% of regenerated fibres at level G and D lack myelin, and ML/AC ratios are normal[20]. In normal regenerating nerves, fibres with no myelin probably represent premyelinated fibres as well as nodes of Ranvier, increased in number because of the shortening of Schwann cell territories along regenerated fibres[21]. The proportions of fibres with Schwann cells that contain no myelin and the MC/AC ratios for the proximal and distal segments are similar for N–T–N and N–N–N nerves. In the grafted segments of N–T–N nerves, however, the proportion of fibres with no myelin is increased sevenfold and ML/AC ratios are only one-third those of normal grafts, indicating that myelin sheaths remain inappropriately thin in relation to axonal diameters (hypomyelination). These findings for N–T–N experiments are reversed in the T–N–T experiments: non-myelin containing Schwann cell percentages and ML/AC ratios are normal in the graft but values in the regenerated distal stump resemble those of the Tr recipient proximal stump.

stumps by 10-0 nylon sutures placed through the epineurium. Three different combinations of nerves were prepared:

- Normal-to-normal nerve grafts (N–N–N). Nerves from normal C57BL/6J mice were grafted into sciatic nerves of 14 non-littermate animals of the same strain. Thus, in this group of mice, normal axons regenerating from the proximal stump of the host should associate with normal Schwann cells in the graft and eventually grow into the distal nerve stump.
- Trembler-to-normal nerve grafts (N–T–N). Segments of nerve from Tr mice were grafted between the stumps of sciatic nerves in six normal C57BL/6J mice. In this group, axons from normal nerves should regenerate across a graft containing Schwann cells from abnormal Tr nerves.
- Normal-to-Trembler nerve grafts (T–N–T). Segments from C57BL/6J mice were grafted into six Tr sciatic nerves. In these animals, Schwann cells from a normal graft should ensheath Tr mouse axons.

To prevent graft rejection, anti-lymphocytic serum (prepared by injecting rabbits with a suspension of 10^9 living thymocytes from C57BL/6J mice[19]) was injected subcutaneously in doses of 0.5 ml twice weekly, beginning on the day of grafting. Two, three and four months after grafting, animals were killed by systemic perfusion of 3% glutaralderhyde and the nerves processed according to standard techniques for quantitative ultrastructural studies[6]. In all experimental groups, no grafts were rejected and axons from the proximal stumps, whether from normal or Tr mice, successfully bridged the graft.

In all experiments, as regenerating axons grew into the grafts, the morphological features of the donor nerve were fully reproduced by the grafted Schwann cells (Figs 1 and 2). The boundaries between normal and abnormal nerve segments were well delineated in these experiments. In N–T–N regenerated nerves, the widespread hypomyelination and absence of myelin, which are the hallmarks of the Tr neuropathy, were duplicated in the grafts and contrasted abruptly with the normal myelin in the recipient nerve stumps. Because Schwann cells proliferate intensely soon after grafting[6,8,11], these findings demonstrate that the manifestations of the gene responsible for the Tr neuropathy are fully expressed by the transplanted Schwann cells and their daughter cells. There was no indication from the T–N–T experiments that axonally mediated or general systemic factors were involved in the pathogenesis of the neuropathy; the Tr abnormality was not induced in normal Schwann cells transplanted into Tr mice.

These experiments have established the feasibility of *in vivo* combinations of axons and Schwann cells from normal and pathological nerves. They have also proved unequivocally that the Tr neuropathy is due to a primary disorder of Schwann cells. Because conditions in the host animal might be expected to modify the behaviour of transplanted Schwann cells, the technique used in the present study should be helpful in elucidating other pathogenic mechanisms; for example, it should be possible to show whether axons or general metabolic factors are responsible for myelin abnormalities in other neuropathies. Observations in these experiments with Tr mice should also be of value in understanding the pathogenesis of the hypomyelination observed in other neuropathies, both in man[22,23] and experimental animals[24].

Finally, our findings provide further proof that Schwann cells in grafts from distal stumps of nerves regenerating in continuity originate from Schwann cells and not by migration from the proximal stumps[6,8,11].

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Spontaneous and evoked potentials from dissociated epithelial cells of *Hydra*

ALTHOUGH intracellular recording from excitable cells has a thirty-year history, such recording from coelenterate tissue has only recently been achieved[1–4]. Mechanical dissociation of previously separated endodermal and ectodermal epithelial layers of *Hydra* produces a preparation permitting intracellular recording from identifiable classes of cells (Fig. 1). Elicited and spontaneous potentials occur in the cells of both epithelial layers. We have recorded small rhythmically occurring pulses from endodermal cells with positive resting potentials; large spikes from ectodermal cells with negative resting potentials, and regularly occurring membrane oscillations from both types of cells.

Cells could be held for up to 3 h, but we generally withdrew the electrode after 20–45 min. Some records only lasted 10 min (see legend of Fig. 2). Cell types were consistently recognisable (Fig. 1) and pulses were obtained from medium-sized endodermal epithelial cells (20–35 μm), having few vacuoles or enclosures; from smaller endothermal interstitial cells (10–15 μm), and from large (>30 μm) ectodermal epithelial cells. Of the cells penetrated only about 5% produced pulses.

Resting potentials in all solutions were positive for almost all endodermal epithelial and interstitial cells (Fig. 2). These ranged from +3 mV to +25 mV, with the majority between +7 mV and +12 mV. Ectodermal cells had negative resting potentials of −2 mV to −25 mV. The five nerve cells penetrated gave resting potentials of −5 mV to −7 mV. During the usual 4–6 h recording session, all intact cells had resting potentials. Whenever successive penetrations yielded no resting potentials, the cells were judged dead and so discarded.

To compare our resting potentials with those in intact animals, intracellular recordings from endodermal and ectodermal cells of intact *Hydra* were undertaken. Animals were immobilised by prior colchicine treatment[9,10] and turned inside out[11] for endodermal cell recordings. Recordings were done in *Hydra* culture solution[12]; endodermal resting potentials ranged between +15 mV and +20 mV (seven cells); ectodermal resting potentials between −2 mV and −8 mV (eight cells).

Resting potentials of dissociated endodermal cells could be driven to negative values by increasing the sodium concentration above 18 mM. However, the cells produced numerous blebs (presumably a result of osmotic stress) and died within an hour or so. Varying the KCl concentration from 0.2 to 3 mM produced no apparent change in resting potentials; but raising the molarity above 3 mM KCl resulted in rapid cell death.

We have tentatively identified three categories of electrical activity: Group 1 pulses, reminiscent of typical junction potentials and of epithelial pulses in *Nanomia*[1]; Group 2 activity representing apparent membrane oscillations, and Group 3 potentials similar to typical action potentials and to epithelial potentials in *Hippopodius*[4]. The most striking characteristics in all three categories is the variability of the pulses, both in amplitude and time course, even within a particular sequence of pulses in the same cell.

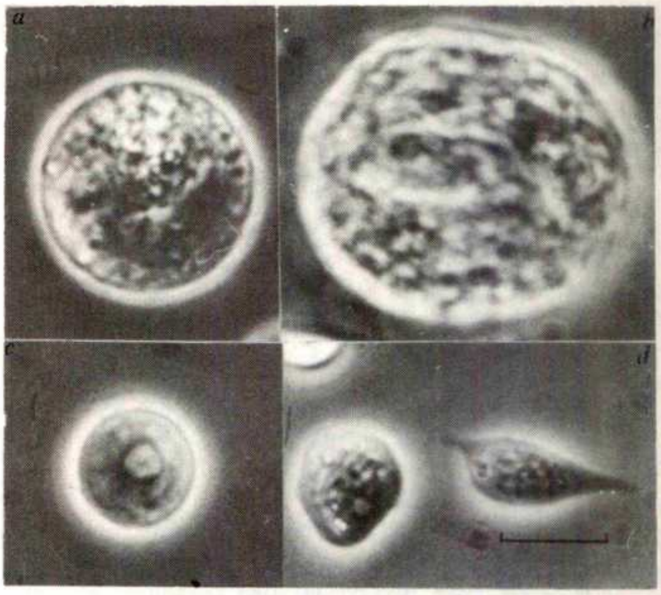

Fig. 1 Dissociated cells. *a*, Endodermal epithelial cell. *b*, Ectodermal epithelial cell containing a glutinant. *c*, Interstitial cell. *d*, Bipolar nerve cell and an interstitial cell. Scales indicate 10 µm. Preparations of dissociated cells from either or both epithelia of *Hydra attenuata* Pall. are made using methods modified from those of Papenfuss and Bodenham[5] and Gierer *et al.*[6] Endodermal epithelio-muscular cells are selectively collected by transecting whole animals at the peduncle, discarding the digestive-cell-bearing upper portions and pipetting the endodermal cells free from the remaining peduncle. Ectodermal cells are gathered by stripping the ectoderm free of endodermal cells in this way and then dissociating the remaining ectodermal shell by gentle pipetting in a centrifuge tube in the recording solution. After pipetting, the cells are filtered through a No. 63 Nitex cloth for additional dissociation and debris removal. Cells are collected by centrifugation for 5 s at top speed, followed by 3 s at medium speed in a clinicial centrifuge. They are then pipetted on to a collagen-covered recording dish on the stage of a Zeiss phase contrast inverted microscope and maintained at a temperature of 20 °C by means of a cooling unit in the microscope stage. Preparations were viewed under phase contrast optics (346×) during recording and both the impaled cell and the recording electrode remained visible throughout.

Group 1 potentials were recorded from single apparently isolated endodermal epithelial and interstitial cells and from cells in cell aggregates (Fig. 2). They often occurred as regular, rhythmic membrane depolarisations having relatively fast rise times (150 ms–2000 ms) and long decay periods (Time constants ranged between 500 ms and 2–4 s.) Amplitudes of the longer lasting pulses were between +2 mV and +5 mV (Fig. 2*a*); those with faster time constants between +0.3 mV and +0.6 mV (Fig. 2*b*).

The larger rhythmic pulses occurred at an average rate of 2 min^{-1} (1.74±0.21 (s.e.) for six cells) (Fig. 2*a*). The smaller pulses often occurred sporadically. They are perhaps best construed as miniature junctional potentials, since both negative-going and positive-going potentials of similar amplitudes and time courses were recorded (Fig. 2*b*).

Other pulses, similar in shape, time course and amplitude occurred as brief responses to stimuli given through the

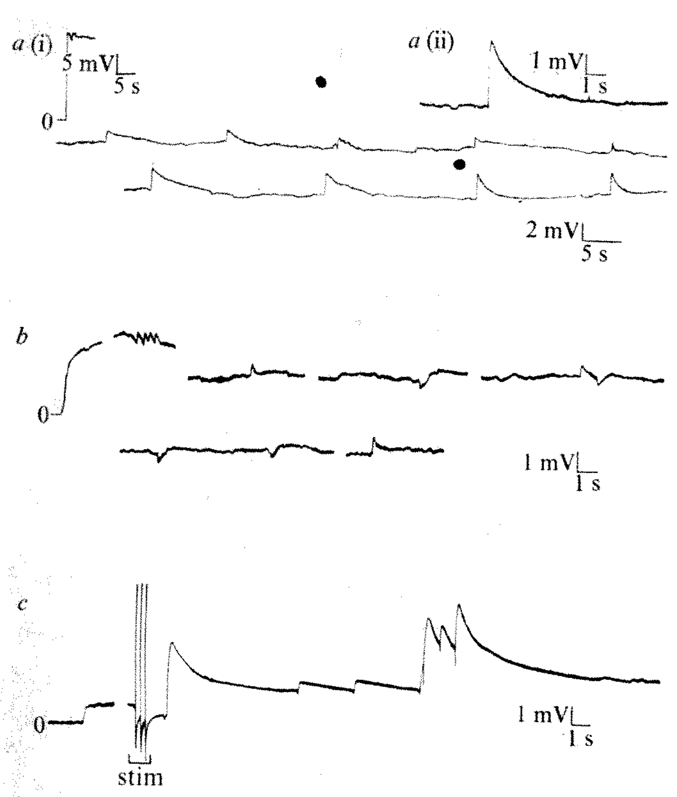

Fig. 2 Group 1 potentials from endodermal cells. Resting potentials are given at the left. Scales at right refer to all preceding pulses. Wave forms in this and all subsequent figures were traced with a pentograph from DC oscillograph records. In *a*(i) spontaneous pulses occur in an isolated cell at a regular rate of about 2 min^{-1}. *a*(ii) is the penultimate pulse enlarged. *b*, Small sporadically occurring spontaneous pulses from an isolated epithelial cell. Both positive and negative-going potentials continued on for 8 min before the electrode was withdrawn. *c*, Positive potentials elicited by a series of stimuli through the recording electrode in an epithelial cell in an aggregate of three cells. The pulses sum or facilitate and culminate finally in a larger, somewhat faster potential. Recordings were made with conventional 3 M KCl filled glass pipettes held by a pair of Jena micromanipulators. Potentials were led to an FET preamplifier with stimulating bridge and simultaneously monitored on a Haer Audio Analyser, a 5103N Tektronix storage oscilloscope and a 7402A Hewlett Packard chart recorder with d.c. amplifiers. Photographs of both cells and oscilloscope traces were taken with Polaroid cameras. A number of solutions were tried as recording media. In different solutions, the salt and sugar concentrations were varied as follows: NaCl from 10 to 15 mM, KCl from 0.2 to 2 mM, $CaCl_2$ from 5 to 8 mM, $MgCl_2$ from 0 to 0.3 mM, $NaHCO_3$ from 0 to 3 mM, EDTA from 0 to 1.4 mM, sugar from 0 to 25 mM. Group 1 recordings were obtained only in sugar-free solutions. Group 2 and 3 recordings in both sugar-containing and sugar-free solutions. All solutions were adjusted to a *p*H of 6.8 to 7.2 and had an osmolarity of between 60 and 70 mosM, the range of the osmotic concentration of the *Hydra* gut[7,8]. A solution was judged usable if the cells remained alive in it for 4 h, if the average value of resting potentials remained unchanged for 2 h and if the cells maintained their tonicity for 4 h. Although cells in sugar-containing solutions met the above criteria, they nevertheless eventually swelled and burst even when no glucose was present.

recording electrode. Those in Fig. 2*c* apparently sum or facilitate. They may indeed have resulted from antidromic stimulation to a nerve impinging on the cell.

Whether any of these decrementing pulses are endogenous or a result of nerve activity is not clear, although many were recorded from cells appearing to be nerve free. We may speculate, however, that since the frequency of the regular pulses falls within the range of the extracellularly recorded endodermal rhythmic potentials[11,13,14] of Passano and McCullough, they are associated with that pacemaker system.

● Group 2 potentials. A series of wave-like membrane oscillations were frequently encountered in endodermal epithelial cells and in some ectodermal cels (Figs 3 and 4).

In endodermal preparations, these were often preceded or accompanied by rapid negative-going potentials, occurring either spontaneously or in response to stimuli given through the recording electrode. The negative transients varied from −0.2 mV to −2 mV and usually disappeared after a while. In one case (Fig. 3*b*) they continued to occur in unison with their concomitant waves for some time; both finally stopping spontaneously. Their significance is not known, although in one recording there was an increase in wave frequency after a succession of these spikes.

These wave-like oscillations may represent a series of junctional potentials as described for other systems. In one preparation (Fig. 3) which had been allowed to dark adapt for 10 min and which was then suddenly illuminated through the condenser, the frequency of waves increased from a rate of 32 min^{-1} in the half minute before the light stimulus to 52 min^{-1} in the half-minute after the shock. Since the recording dish was on a microscope cooling stage, and the response to sudden illumination instantaneous, it it unlikely that this was actually a heat response. An increase in pulse rate similarly occurs in the extracellularly recorded rhythmic potential system when the bases of intact *Hydra* are stimulated with light[14]. It is quite possible, therefore, that this response may in fact be due to nerve activity.

Alternatively these oscillations may be a function of the membrane itself, since they can occur when spikes from a previously spiking ectodermal cell fail; the oscillations apparently replacing the spikes (Fig. 4). Waves in ectodermal cells also seem capable of developing into spikes (see below).

● Group 3 potentials were large, rapid spikes of +35 mV to +200 mV which could occur individually or in bursts (Fig. 4*a*). These arose spontaneously from cells, approximately 1.5 times the size of medium epithelial cells, which we classified as ectodermal epithelial cells (Fig. 1*b*).

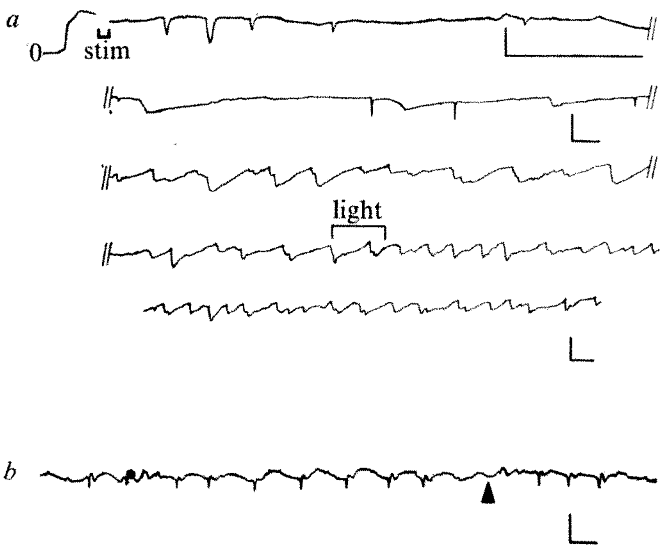

Fig. 3 Group 2 potentials from endodermal cells. Resting potential is on the left. Scales at right refer to preceding pulses (vertical axis equals 1 mV, horizontal axis equals 1 s). *a*, Rapid negative-going pulses elicited by stimuli through the recording electrode, followed by spontaneous oscillations of the membrane which increased in frequency after the first 5 min of recording and then slowed down again. A light shock given 53 min after penetration causes an increase in the oscillatory frequency in the same cell. Deletions from the record greater than 10 min are indicated by slashes. The absence of a slash indicates that the record is continuous. *b*, Spontaneous negative potentials accompanying membrane oscillations in another isolated epithelial cell. Arrow indicates an instance where the wave and spike occurred out of phase.

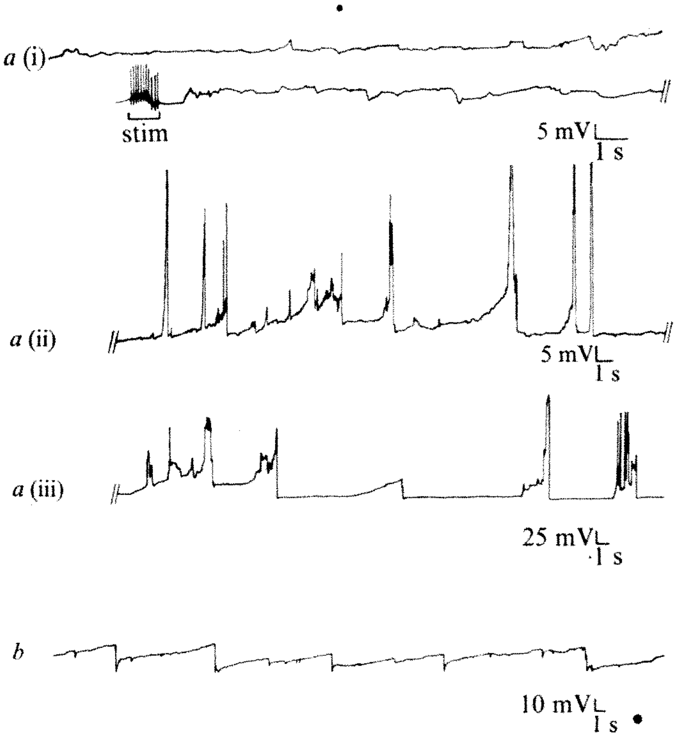

Fig. 4 Group 3 potentials from ectodermal cells having negative resting potentials. *a*(i), Spontaneously occurring membrane oscillations which increase in regularity in response to stimuli through the recording electrode. *a*(ii), *a*(iii), Large spikes occurring singly or in bursts in the same cell. In *a*(iii) the membrane does not always reach threshold. Deletions from the record greater than 8 min are indicated by slashes. The absence of a slash indicates that the record in continuous. *b*, Oscillations of the membrane potential recorded from a cell in an aggregate, which have replaced previously occurring spikes after 2.5 h of spontaneous spiking activity.

The spikes usually arose from gradual depolarisations of the mebrane and often had small after-hyperpolarisations. They frequently displayed a distinct rhythmicity (Fig. 4*a*(ii) and *a*(iii)).

As before, these too varied greatly in amplitude and time course. (Durations ranged between 300 ms and 900 ms.) This was so even for a series of potentials in the same burst. Irregularly shaped action potentials of various amplitudes are reported for other systems[15,16], as well as for coelenterates[2].

In one case, spikes arose from successive membrane oscillations which increased in frequency upon stimulation through the recording electrode. These grew into +15 mV depolarisations carrying spikes at their crests; they later became huge individual potentials greater than +200 mV. In another, Fig. 4*b*) the spikes were replaced after 2.5 h by rhythmic oscillatory waves having small negative-going pulses interspersed among them. (These were perhaps thus inhibitory junctional potentials.) The oscillations were reminiscent of those recorded from endodermal cells (Fig. 3).

Here too, we are not certain whether these pulses are endogenous or the result of nerve activity. But since the membrane potential itself often seemed to become unstable, exhibiting wave-like spontaneous depolarisations comparable to those known for vertebrate smooth muscle[17], it is conceivable that even if this activity were initially induced by nerves, once the cell had become active, it could continue to produce spikes endogenously. A relationship of these spikes to the extracellularly defined contraction burst system[18] is possible, but remains to be investigated.

In summary, then, although it is too early to classify these potentials with certainty, or to assign them to specific cell types, or to say whether they are endogenously produced by the cells from which they are recorded, it is nonetheless possible to offer tentative suggestions. We would like to propose that, on the basis of their distinct regularity, the rhythmically occurring Group 1 pulses are either junctional potentials which induce the endodermally conducted rhythmic potentials[13], or themselves underlie these potentials and that the oscillatory Group 2 membrane fluctuations may be a property common to both sorts of excitable epithelial cells. This suggests that *Hydra* epithelial cells possess properties typical of spontaneously active cells whose membranes are capable of prolonged periods of intermittent endogenous depolarisations[17,19]; even if these depolarisations are initially induced by nerve activity.

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Response of cricothyroid muscles to frequency-modulated sounds in FM bats, *Myotis lucifugus*

BATS of the species *Myotis lucifugus* use short frequency-modulated (FM) ultrasonic pulses for echolocation. The duration, repetition rate and frequency of these pulses vary from time to time depending upon whether the animal is searching for, approaching or actually capturing its prey. The bat emits 2–5 ms pulses at a rate of 10–20 s^{-1} with the frequency of each pulse sweeping approximately one octave from 80 to 40 kHz during the searching and cruising flight; it produces 0.3–1.0 ms pulses at a rate of 150–250 s^{-1} with a downward-sweep FM from 40–20 kHz immediately before avoidance of obstacles or capture of a prey[1,2]. Among the laryngeal muscles of the bat, the cricothyroid muscle (CTM) in particular, is highly developed and is essential in producing the intense orientation pulses[1,3]. The CTM not only discharges action potentials before the vocalisation[3–5], but also during the acoustic stimulus[6,7]. The latter phenomenon is called the acoustic laryngeal muscle reflex and might serve as a negative feedback to stabilise the performance of the vocalisation system[6]. Comparative studies of the properties of the middle ear and laryngeal muscles revealed significant differences between these two muscles (refs 6 and 7 and P.H.-S.J., J.O., and N. Suga, in preparation). We found that CTM fibres were more sensitive to 4 ms FM signals sweeping downward with a range of 10 or 20 kHz across their best frequencies (BFs) than to pure tones.

Seven *M. lucifugus* were anaesthetised with ether for surgery. The exposed dorsal surface of the skull of a bat was mounted with the flat head of a 1.8 cm long nail with glue and dental cement and the animal was placed ventral side up in a body-sized box made of wire mesh. This box was suspended with rubber bands at its four corners so that it absorbed the body movement from the bat. The head of the animal was immobilised by fixing the shank of the nail into a metal rod with a set screw. The laryngeal muscle was then exposed. After the animals had recovered from the anaesthesia, the action potentials of single CTM fibres were recorded with sharpened and coated tungsten wires from the conscious animals and were displayed on the screen of an oscilloscope. An indifferent electrode of silver wire was placed on the nearby subcutaneous tissue. The electronic instruments used to generate acoustic stimuli were basically the same as described elsewhere[8,9]. Briefly, tone pulses with duration of 4 ms and 0.5 ms rise–decay time were delivered at a rate of 1.5 s^{-1} from a condenser loudspeaker placed 75 cm in front of the animal. The loudspeaker was calibrated with a Brüel and Kjaer ¼ inch microphone placed at the bat's ears and its output was expressed in dB SPL referred to 2×10^{-4} dyne/cm^2 root mean square. Frequency-modulated signals sweeping at a range of 5, 10, 20 and 30 kHz respectively during the 4 ms duration were generated from a VCG Wavetek (model 186) driven by a sawtooth generator. The sweep range of FM pulses were carefully adjusted so that their centre frequencies were always at the BF of each investigated fibre. The discharge pattern of 52 CTM fibres studied were always tonic-on responding, that is they discharged many action potentials during the stimulus. The shortest latency of this acoustic cricothyroid muscle (CTM) reflex was 6.2 ms which is about 3 ms longer than that of the middle-ear muscle (MEM) reflex. This suggests that acoustic CTM reflex may have a longer neuronal chain than MEMs. The tuning curves of the 52 CTM fibres were of simple triangular shape and were very similar to one another. The best frequencies of these 52 fibres were between 28 and 43 kHz with an average value of 39±1.6 kHz. These tuning curves had sharper slopes both on the high and low frequency sides of the BF and thus had higher Q-10 dB values than that of the MEM fibres. The lowest minimum threshold of the CTMs is about 20 dB higher than the MEMs. When FM signals were delivered, the thresholds of the response were either higher or lower than the thresholds to pure tones depending upon the sweep range and sweep direction of the FM. On the average, a 10 kHz range of downward-sweep FM was 5 dB more effective than a pure tone, but a 20 kHz range of downward-sweep FM was only slightly better. A 10 or 20 kHz range of upward-sweep FM was usually not better than a pure tone. Furthermore, a 5 kHz or 30 kHz range FM signals sweeping either direction was always poorer than a pure tone. In Fig. 1, we present a representative tuning curve of a CTM fibre and its thresholds to FM signals at 10 and 20 kHz frequency sweep ranges. This fibre responded only in a small frequency band between 45 and 29 kHz. Its tuning curve shows a slope of 240 dB per octave and 140 dB per octave at its high and low frequency sides respectively. These slopes are much sharper than those obtained from the MEM fibres[7,10]. Note that the threshold for 10 kHz range downward-sweep FM is lower than pure tone and the equivalent upward-sweep FM. The threshold difference between the 10 kHz upward and downward sweep FM pulses was as large as 10 dB. The threshold for the 20 kHz range downward-sweep FM was almost the same as for the pure tone of 40 kHz, but the threshold for the equivalent 20 kHz upward-sweep FM was 9 dB less effective. The minimum threshold for this CTM fibre was 54 dB SPL.

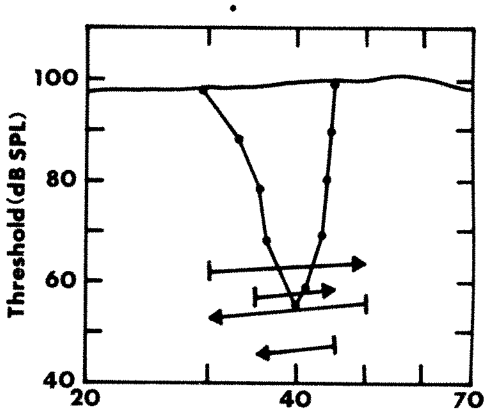

Fig. 1 The excitatory response area (solid line, filled circles) measured by 4-ms tone pulses with 0.5 ms rise and decay times and thresholds for FM tone pulses (arrows) of a representative CTM fibre. The ordinate and abscissa represent the stimulus amplitude in dB SPL (re 0.0002 dyne cm^{-2}) and frequency in kHz respectively. The uppermost solid line is a frequency response curve of the condenser loudspeaker used to deliver tone pulses. The arrows represent the directions of frequency sweeps of the 4-ms FM pulses. The different ranges of the FM sweep pulses are reprseented by the different lengths of the arrows and the thresholds of this CTM fibre for them are shown by the vertical positions of the arrows (see text for the properties of this fibre).

Fifty-one of the 52 CTM fibres studied had a threshold higher than 50 dB SPL and a narrow frequency response band between 45 and 25 kHz. Because of the frequency content of a bat's orientation sounds[1,2] and the highly directional sensitivity of its echolocation system[11] the acoustic CTM reflex of a bat will generally be evoked only by the sounds emitted by the bat itself or another bat which is flying toward this bat during the approaching or terminal phases. Returning echoes during the terminal phase may also evoke the reflex because their sound amplitudes may be higher than the threshold of this reflex. This speculation is supported by the present results that 10 or 20 kHz range of downward-sweep FM signals mimicking the natural orientation sounds during the final phase of flight were more effective stimuli than the corresponding upward-sweep FM signals or pure tones. An asymmetrical lateral inhibition at the low frequency side of the excitatory response area as that found in the collicular neurones and cortical neurones[12] may also exist in the CTM fibres which causes these fibres to be less sensitive to an upward FM stimulus than to a downward-sweep one.

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Suppression of noradrenaline synthesis in sympathetic nerves by carbidopa, an inhibitor of peripheral dopa decarboxylase

IT is generally believed that noradrenaline synthesis in postganglionic sympathetic nerves can be suppressed by drugs which inhibit the activities of tyrosine hydroxylase or dopamine β-oxidase[1,2], but not by inhibition of aromatic l-amino acid decarboxylase (AAAD; dopa decarboxylase). We present here evidence that the administration to rats of carbidopa (MK-486; α-methyl-L-dopa-hydrazine), a peripherally acting inhibitor of AAAD[3], not only blocks the decarboxylation of exogenous L-dopa (for example, in capillary endothelia within the central nervous system, liver, and kidney[4,5]), but also suppresses the decarboxylation of endogenous dopa formed from ^{3}H-tyrosine within cardiac sympathetic nerve terminals. As a consequence, the drug inhibits noradrenaline synthesis in this tissue.

Seven separate experiments, all yielding similar observations, were performed, using 122 normotensive male Sprague–Dawley rats (150–200 g) and 60 spontaneously hypertensive (SHR) male Wistar–Okamoto rats (Charles River Laboratories). In each experiment, groups of 4–10 rats received carbidopa (3.2–100 mg kg^{-1}, intraperitoneally, suspended in 0.05 N HCl or 0.9% saline) or its diluent, followed in 60 min by ^{3}H-tyrosine (80–100 μCi, in a total volume of 0.1–0.3 ml, intraperitoneally, specific activity 53.3 mCi μmol^{-1}, New England Nuclear). Animals were decapitated 60 min later and hearts were taken for assay of tyrosine[6], ^{3}H-tyrosine, ^{3}H-dopa and ^{3}H-catecholamines (including both ^{3}H-dopamine and ^{3}H-noradrenaline)[7,8]. (In preliminary studies we observed that the rates at which cardiac sympathetic terminals accumulate ^{3}H-dopa and ^{3}H-catecholamines are fairly linear during the 60-min interval used in these studies.)

Table 1 Effect of carbidopa on accumulation of ^{3}H-catechols in cardiac sympathetic nerves

	Control	Carbidopa
Tyrosine (μg g^{-1})	13.1 ± 0.42	28.9 ± 1.6*
^{3}H-tyrosine, specific activity ((d.p.m. μmol^{-1}) × 10^5)	7.2 ± 0.79	12.7 ± 1.1*
^{3}H-dopa (d.p.m. per g tissue)	173 ± 29	380 ± 29*
^{3}H-dopa/specific activity of ^{3}H-tyrosine ((μmol per g tissue) × 10^{-4})	8.3 ± 1.5	9.3 ± 6.1
^{3}H-catecholamines (d.p.m. per g tissue)	261 ± 26	155 ± 33†
^{3}H-catecholamines/spec. act. of ^{3}H-tyrosine ((μmol per g tissue) × 10^{-4})	11.5 ± 1.3	4.7 ± 1.0*

Groups of 20 male Sprague–Dawley rats received carbidopa (100 mg kg^{-1}, intraperitoneally) or its diluent, and, after 60 min, ^{3}H-tyrosine; the animals were killed 60 min later. Data are given as means ± s.e.m.

*$P < 0.001$ differs from control values.

†$P < 0.02$ differs from control values.

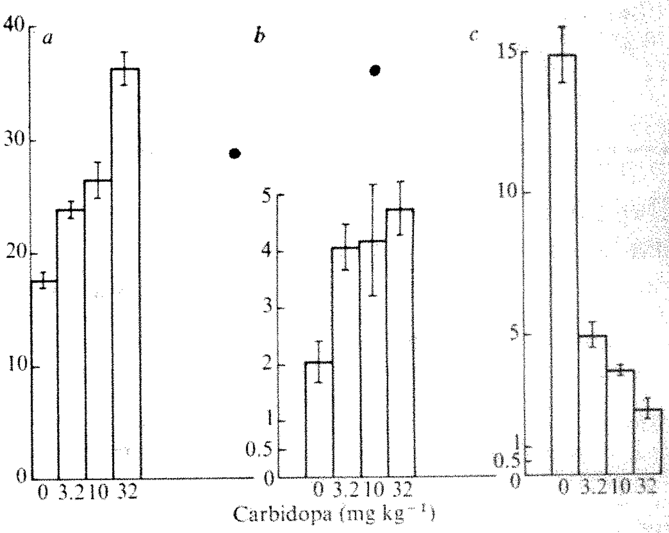

Fig. 1 Effect of carbidopa on the accumulation of ^{3}H-dopa and ^{3}H-catecholamines in hearts of SHR rats. Groups of eight rats were killed 120 min after receiving carbidopa or its diluent intraperitoneally, and 60 min after receiving ^{3}H-tyrosine (80 μCi; specific activity 53.3 mCi μmol^{-1}) intraperitoneally. Data are presented as ^{3}H-dopa (or ^{3}H-catecholamines) divided by the specific activity of cardiac ^{3}H-tyrosine. Vertical bars represent s.e.m. *a*, Tyrosine (μg g^{-1}); *b*, ^{3}H-dopa/specific activity of ^{3}H-tyrosine (μmol g^{-1} × 10^{-3}); *c*, ^{3}H-catecholamines/specific activity of ^{3}H-tyrosine (μmol g^{-1} × 10^{-4}).

In all our experiments, carbidopa at least doubled the concentration of tyrosine in cardiac homogenates (Table 1), and caused an even greater increase in cardiac ^{3}H-tyrosine. Thus it increased the specific activity of the ^{3}H-tyrosine present in the hearts (Table 1). For this reason, data on the amounts of ^{3}H-dopa and ^{3}H-catecholamines accumulating in the hearts were expressed both as d.p.m. per g of tissue and as μmol per g divided by the specific activity of the cardiac ^{3}H-tyrosine. Regardless of the method of data calculation used, the effects of the drug were the same, that is, it increased accumulation of ^{3}H-dopa and decreased that of ^{3}H-catecholamines. In experiments with normotensive rats (Table 1), concentrations of ^{3}H-dopa increased by 120% ($P<0.001$) and concentrations of ^{3}H-catecholamines decreased by 40% ($P<0.02$) when expressed as d.p.m. per g of tissue; these changes were +12% (not statistically significant) and −59% ($P<0.001$) when corrected for the specific activity of the ^{3}H-tyrosine.

SHR Wistar–Okamoto rats were found to be highly sensitive to the carbidopa inhibition of AAAD in cardiac sympathetic nerves: a concentration of the drug as low as 3.2 mg kg^{-1} almost doubled the accumulation of ^{3}H-dopa ($P<0.001$), and reduced ^{3}H-catecholamine accumulation by 60% ($P<0.001$) (Fig. 1). Larger doses of carbidopa were even more effective in these animals.

Administration of carbidopa reportedly has no effect on the AAAD in brain neurones of intact animals in the dose range used in our study[3,9,10]. In preliminary studies, we also failed to observe any suppression of brain ^{3}H-catecholamine synthesis (d.p.m. per g of tissue) in rats given ^{3}H-tyrosine and carbidopa. Thus, as far as we know, carbidopa may constitute the first clinically useful agent that suppresses noradrenaline synthesis in sympathetic neurones without significantly modifying brain catecholamine synthesis. Its effect on sympathetic nerves might underlie the ability of carbidopa to potentiate the antihypertensive actions of chemically diverse agents in spontaneously hypertensive rats[11]. Carbidopa might have value in the therapy of diseases involving peripheral catecholamines (or serotonin) in which the physician does not wish to modify brain function.

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Dopamine is a monoamine oxidase B substrate in man

DURING the past decade, the central neurotransmitter, dopamine (DA), has been much studied for it is known to be involved in certain human disease states, Parkinsonism[1] preeminently, and rather more inferentially, schizophrenia[2]. DA is metabolised by monoamine oxidase (MAO)[3], which catalyses the oxidative deamination of a wide range of monoamines[4,5], including the neurotransmitters noradrenaline (NA) and 5-hydroxytryptamine (5-HT), and other amines such as tyramine, tryptamine and phenylethylamine (PEA). On the basis of animal studies with the inhibitors, clorgyline[6], and later deprenil[7], MAO has been classified into two types, A and B. By definition, type A is selectively inhibited by clorgyline and type B by deprenil. In general, type A acts on 5-HT[6] while type B prefers PEA[8]. In the rat, DA is preferentially deaminated by MAOA[9–11]. It thus seemed paradoxical that, in the treatment of Parkinsonism, addition of the MAOB inhibitor, deprenil, to a DA-generating drug combination should produce further therapeutic benefit[12]. In this report, we reconcile these apparently conflicting observations by demonstrating that, as far as DA oxidation is concerned, man may be different from rat: in two sites we have investigated, platelet and brain, DA is preferentially deaminated by an enzyme with the characteristics of MAOB.

Table 1 MAO activity of human platelets with PEA and DA as substrates

Subject	PEA	DA	DA:PEA
1	2.9	3.6	1.2
2	5.7	6.1	1.1
3	16.7	15.4	0.9
4	11.0	14.4	1.3
5	9.8	4.8	1.0
6	4.4	4.4	1.0
7	9.2	9.0	1.0
8	2.2	1.8	0.8
9	11.4	14.2	1.2
10	9.3	8.9	0.9
11	8.8	8.9	1.0
12	14.9	14.3	1.0
13	10.2	10.5	1.0

Activities are expressed as nmol of product formed per mg of protein per 30 min at 37 °C.

One way to trace the number and specificity of independent catalytic sites of MAO, which seem to vary both for tissue and species, is to do cross-inhibition experiments: White and Wu[13] used a wide range of substrates with human brain MAO and found extensive cross inhibition, apart from with PEA and 5-HT where there was none. Another approach, which we have used, is to see how activity varies when different substrates are used, whether with the same tissue from different individuals or with samples taken from different regions of the same individual. If only one catalytic centre deaminates a particular group of substrates, the ratio of activities should be constant.

We assayed MAO radiometrically by a cation exchange resin method for PEA[14], and an extraction procedure for DA and 5-HT[13]. The only modifications of existing methods were of scale: 100 μl of buffer was used with 20 μl of homogenate and 20 μl of substrate. The specific activities of the substrates were also increased fivefold and the final concentrations of DA or 5-HT in the incubation mixture was 150 μM, and of PEA, 50 μM. Incubations were carried out for 30 min at 37 °C and blanks with substrate but no enzyme and with boiled enzyme were incubated with each batch. The reaction was shown to proceed linearly for at least 30 min. Serial dilutions of clorgyline and deprenil in water were freshly prepared: 20 μl of each was added to 100 μl of buffer and 20 μl of enzyme suspension and left at room temperature for 30 min before starting the reaction with ^{14}C-DA. Platelets were collected from 10-ml venous blood samples collected into 10 ml of 2% EDTA in normal saline[15]. Normal human brains, obtained up to 48 h after death, were dissected, and separate regions were stored at −40 °C. Mitochondria and synaptosomes were prepared by differential centrifugation[16].

Human platelets have a type B enzyme which actively deaminates PEA[17]. This enzyme has low activity towards 5-HT and is inhibited by deprenil rather than clorgyline. Table 1 shows the activity of platelet MAO towards PEA and DA in samples chosen with widely different PEA-oxidising ability from different individuals. The PEA: DA ratio is constant whether absolute activities are high or low and the two activities are strongly correlated (r= 0.96). This indicates that the catalytic activities are controlled together and are probably located on the same molecule. The platelet enzyme is as active with DA as with PEA as substrate.

Table 2 shows the results of an experiment in which PEA, 5-HT and DA were used as substrates for MAO from several regions of a single human brain. Here the PEA: 5-HT ratio varied greatly from 16 in hypothalamic synaptosomes to 0.7 in substantia-nigral mitochondria, providing support for the existence of independent catalytic sites for 5-HT and PEA. There is clearly DA-oxidising activity in the regions of low 5-HT activity and in this brain it parallels that of PEA. In this brain there was a low 5HT: PEA ratio in the synaptosomal fraction.

We next examined the effects of clorgyline and deprenil

Table 2 MOA MAO activities in different regions of human brain with PEA, 5-HT and DA as substrates

Region	PEA	5-HT	DA	DA:PEA	PEA:5-HT	DA:5-HT
Synaptosomes						
Thalamus	25	9.1	31	1.2	2.7	3.4
Hypothalamus	72	4.5	60	0.8	16.0	13.0
Frontal cortex	13	4.4	11	0.8	3.0	2.5
Substantia nigra	49	29.0	55	1.1	1.7	1.9
Caudate	22	5.6	25	1.1	4.0	4.5
Mitochondria						
Thalamus	92	55	98	1.1	1.7	1.8
Hypothalamus	104	113	82	0.8	0.9	0.7
Frontal cortex	49	52	48	1.0	0.9	0.9
Substantia nigra	94	136	101	1.1	0.7	0.7
Caudate	71	44	73	1.0	1.6	1.6
Nucleus accumbens	75	35	67	0.9	2.1	1.9

Activities are expressed as nmol of product formed per mg protein per 30 min at 37 °C. Assays were carried out in duplicate.

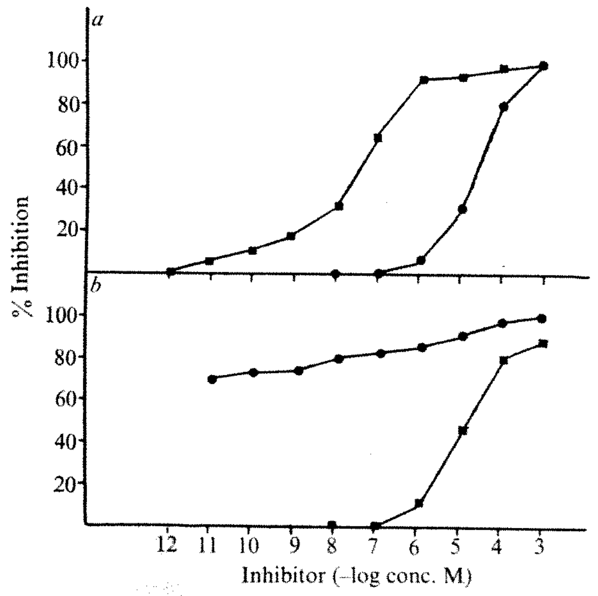

Fig. 1 Percentage inhibition of MAO activity plotted against concentration of deprenil (■) or clorgyline (●) in the incubation mixture, with DA as substrate. *a*, Human caudate mitochondria. Each point is the mean of values obtained using mitochondria from three brains. *b*, Rat brain. Each point is the mean of values obtained with homogenates from two brains.

on the DA-oxidising activity of human caudate mitochondria obtained from three brains. In each case it was selectively inhibited by deprenil (Fig. 1*a*). We also confirmed the finding[11] that the two inhibitors have the reverse effect on rat brain (Fig. 1*b*). Figure 2 shows the effect of deprenil on MAO from human putamen mitochondria, using 5-HT, PEA and DA as substrates. It demonstrates that the pattern of DA-oxidising activity resembles that of PEA rather than that of 5-HT. At low concentrations of deprenil, PEA and DA are selectively inhibited compared with 5-HT. The DA curve does not exactly follow that for PEA and both extend over several orders of magnitude. It may be that both substrates are oxidised by more than one form of MAO and that some of the DA-oxidising activity is also associated with that of 5-HT.

Although one cannot define the number of forms of MAO catalysing the oxidation of DA in the human striatum from these results, it is possible to draw the following conclusions. In man, DA oxidation can be independent of 5-HT-oxidising activity, a finding demonstrated most clearly in the platelet. In the human striatum, it is inhibited by smaller concentrations of deprenil than of clorgyline, resembling PEA-oxidising activity rather than that of 5-HT. This DA-oxidising mechanism seems to differ from that of rat brain although this tissue is inhomogeneous, an area with anomalous properties having been detected in the circumventricular region[18]. Other anomalous findings in rat[19] and beef[20] heart make the conclusion inescapable that MAO is more complex than the simple A/B classification suggests.

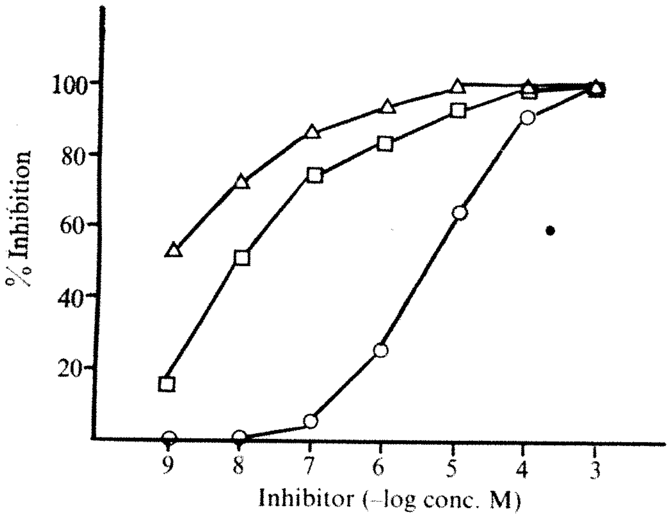

Fig. 2 Percentage inhibition of human putamen mitochondrial MAO activity plotted against concentration of deprenil in the incubation mixture with 5-HT (○), DA (□) and PEA (△) as substrates. Each point is the mean of values obtained by using mitochondria from two brains.

Whether the human enzyme shows similar variations from tissue to tissue awaits further investigation. As implied earlier, however, we have shown that the striatal enzyme has properties such that the addition of deprenil to a central dopamine-generating drug regimen[12] now seems to be a rational treatment of Parkinsonism.

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Concanavalin A as a carrier of daunomycin

ONE possible approach for increasing the effectiveness of anti-tumour drugs would be to find methods of altering their distribution in the body to increase their local concentration at the tumour cell sites. Ghose *et al.* have reported the suppression of Ehrlich ascites carcinoma in mice after administration of chlorambucil allegedly bound to anti-tumour antibodies[1]. This immunochemical approach to cancer has great potential, but the production of highly specific antibodies suitable for clinical use may prove to be a major practical problem.

Concanavalin A agglutinates and kills tumour cells *in vitro* under conditions where it has no such effect on normal cells. Administrated to mice it kills KHT fibrosarcoma tumour cells and, less markedly, AKR lymphoma cells at doses where it has no effect on numbers of normal hematopoietic colony forming cells[2]. The striking selectivity of con A prompted us to explore the possibility of using the drug as a carrier of daunomycin instead of specific anti-tumour antibody. We describe here the preparation of the conjugate and its striking anti-tumour activity.

Periodate oxidation of daunomycin was performed to cleave the bond between C-3 and C-4 of the sugar moiety, producing carbonyl groups capable of reacting with NH_2 groups on the con A and the resulting Schiff base linkages were reduced with $NaBH_4$ to form a peptide bond. Daunomycin 40 mg ml^{-1} in 1 ml of phosphate-buffered saline (PBS) was mixed with a slight molar excess of 0.1 mM $NaIO_4$ and incubated for 1 h at room temperature in the dark. Glycerol was then added to a final concentration of 0.05 M to consume excess periodate. The solution of oxidised drug was mixed with 1 ml of con A 15 mg ml^{-1} in 0.15 M potassium phosphate buffer (*p*H 9.5) and incubated for 1 h at room temperature. $NaBH_4$ was then added to a final concentration of 0.3 mg ml^{-1}, and the reaction was allowed to proceed for 1 h at 37 °C[3]. Free and bound drugs were separated by gel filtration chromatography, using Sephadex G-100. The degree of substitution was estimated by the drug absorbance at 495 nm and protein concentration was assessed by optical density at 280 nm. The extent of substitution was 2 to 3 mol daunomycin per mol con A. Concanavalin A–daunomycin conjugate was dialysed against PBS for several hours and *in vivo* studies were performed within 24 h. Agglutination assays for sheep erythrocytes were performed in a final volume of 250 μl of PBS at a cell concentration of 1×10^8 cells per ml. Con A and con A–daunomycin conjugate equally agglutinated the erythrocytes at the minimum concentration of 2 μg ml^{-1}. SDS polyacrylamide disc gel electrophoresis of the conjugate revealed one protein band with molecular weight about 26,000.

Table 1 Survival of BDF_1 mice bearing L_{1210} cells

Daily treatment (i.p.) (days 1–5)	Mean survival time (d)
Control	7.0
Daunomycin (0.4 mg kg^{-1})	7.4
Con A (8 mg kg^{-1})	7.2
Con A (8 mg kg^{-1}) + Daunomycin (0.4 mg kg^{-1})	7.2
Con A–daunomycin (8 mg kg^{-1})	11.4
Con A–daunomycin (8 mg kg^{-1}) + Hydrocortisone (2 mg kg^{-1})	11.2

To show the effectiveness of the conjugate as an antitumour agent *in vivo*, several groups of five BDF_1 mice were inoculated with 1×10^5 live L_{1210} cells intraperitoneally and BALB/c mice were inoculated with 2.5×10^6 live Ehrlich ascites carcinoma cells intraperitoneally. After 24 h the mice received the first of five daily injections of the conjugate and control preparations. Table 1 and 2 show the results of such experiments. The greatest increase in survival was obtained when the conjugate was given and Ehrlich ascites carcinoma cells were more sensitive to the conjugate than L_{1210} cells.

Table 2 Survival of BALB/c mice bearing EAC cells

Daily treatment (i.p.) (days 1–5)	Mean survival time (d)
Control	23.6
Daunomycin (0.1 mg kg^{-1})	25.6
Con A (2 mg kg^{-1})	27.2
Con A (2 mg kg^{-1}) + Daunomycin (0.1 mg kg^{-1})	27.8
Con A–daunomycin (2 mg kg^{-1})	all alive
Con A–daunomycin (2 mg kg^{-1}) + Hydrocortisone (1 mg kg^{-1})	all alive

One volume of tumour cell suspensions (1×10^7 cells per ml of PBS) were mixed with 1 volume of concanavalin A solution (375 μg per ml of PBS). After manual shaking, the mixtures were incubated at room temperature for 1 h and the cell aggregates consisting of more than three cells were counted. The aggregated cells of Ehrlich ascites carcinoma and L_{1210} were 36 and 10 per 200 cells respectively. There may exist some relationship between cell agglutinability by con A *in vitro* and sensitivity to the conjugate *in vivo* although the precise mechanism is not known.

Con A is a potent mitogen for mouse lymphocytes *in vitro*, therefore the conjugate may be toxic for mouse lymphocytes. Steroid hormone inhibits T cell response to mitogen. Intracutaneous injection of con A induces the haemorrhagic Arthus-like lesions[4] which can be inhibited with steroid hormone. Hydrocortisone was given simultaneously with the conjugate to inhibit lymphocyte damage and Arthus-like lesions and did not inhibit the antitumour activity of the conjugate (Tables 1 and 2).

The con A–daunomycin conjugate may be sufficiently toxic and selective in its effect to be potentially useful in *in vivo* therapeutic studies.

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Recognition sequence of specific endonuclease *Bam*HI from Bacillus amyloliquefaciens H

MANY specific endonucleases (restriction endonucleases) have been isolated and recognition sequences have been determined for a number of them[1]. The isolation of a new specific endonuclease, *Bam*HI, from *Bacillus amyloliquefaciens* H has recently been described[2]. We have determined the recognition sequence of *Bam*HI and find that it cleaves the two-fold rotationally symmetric sequence

↓
5′-G-G-A-T-C-C-3′
3′-C-C-T-A-G-G-5′
↑

at the positions indicated by the arrows generating fragments with cohesive termini.

The *Bam*HI used for these experiments was purified and used as described previously[2]. This endonuclease gives relatively few fragments from most viral DNAs and, to ensure a statistically significant sampling, parallel experiments were carried out on bacteriophage λ DNA (five sites)[2], adenovirus-(Ad-2) DNA (three sites)[2] and Φ 80suIII+ DNA (eight sites). The experiments described in detail used Ad-2 DNA, which

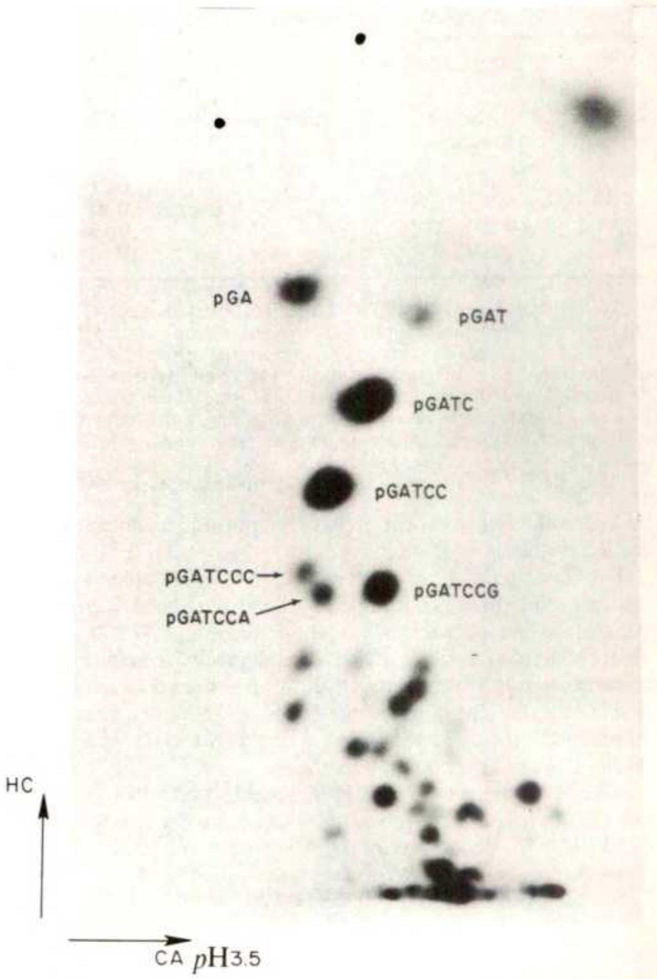

Fig. 1 Pancreatic DNase digestion products of 5′-terminally labelled *Bam*HI fragments. Ad-2 DNA (10 μg) was incubated in a reaction mix (100 μl) containing 6 mM Tris-HCl pH 7.9, 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol and 10 units *Bam*HI for 1 h at 37 °C. Alkaline phosphatase (5 μg) was added, the volume increased to 150 μl by adjusting to 50 mM Tris-HCl, pH 8.9, 10 mM $MgCl_2$ and incubation continued for a further 1 h at 37 °C. Following extraction with phenol (4 × 150 μl), the DNA fragments were precipitated with EtOH and recovered by centrifugation (20 min, 100,000g). The DNA was phosphorylated in a reaction mix (100 μl) containing 50 mM Tris-HCl pH 9.5, 10 mM $MgCl_2$, 5 mM dithiothreitol, 5% glycerol, 10 μM γ-^{32}P-ATP (specific activity 1,000 $\mathrm{C_i mmol^{-1}}$) and 10 units polynucleotide kinase (Biolabs) and incubated for 1 h at 37 °C. Unreacted γ-^{32}P-ATP was removed by passage over a Sephadex G-50 column run in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.9, 0.001 M EDTA. Labelled *Bam*HI fragments were recovered from the void volume by precipitation with EtOH (2 volume) and centrifugation (20 min, 100,000g). The DNA was then incubated in a reaction mix (20 μl) containing 0.1 M NaOAc pH 5.0 and pancreatic DNAse (5 μg) for 30 min at 37 °C, and lyophilised. This reaction mixture was redissolved in H_2O (3 μl) and fingerprinted using electrophoresis on cellulose acetate at pH 3.5 in the presence of 7 M urea for the first dimension followed by homochromatography[5] on a thin layer of DEAE-cellulose (1:10) eluting with homomix VI[6] for the second dimension. The 5′-terminal oligonucleotides were detected by autoradiography, and their sequences deduced from mobility shifts and also as indicated in the text. Oligonucleotides, used for further digestion, were eluted from the thin layer plate in the conventional way[5].

cannot be labelled with polynucleotide kinase prior to restriction endonuclease cleavage (R. J. R., unpublished results), thus avoiding problems of interpretation due to labelling at the ends of the viral genome. The sequences present at the 5′ termini of the *Bam*HI fragments were analysed as follows: Ad-2 DNA was digested with *Bam*HI, the 5′-terminal phosphate was removed by treatment with alkaline phosphatase (an obligatory step) and replaced by a ^{32}P-phosphate using polynucleotide kinase[3] and γ-^{32}P-ATP[4]. The labelled *Bam*HI fragments were digested with pancreatic DNAse and the resulting oligonucleotides fractionated by electrophoresis on cellulose acetate at pH 3.5 in the presence of 7 M urea, followed by homochromatography[5] using homomix VI[6]. An autoradiograph of the resulting fingerprint is shown in Fig. 1.

Table 1 Identification of the 5′-dinucleotide present after cleavage with *Bam*HI

Dinucleotide	R_B (System 1)	R_Y (System 2)
pGA	1.62	0.59
pGC	1.76	0.47
pGG	1.10	0.69
pGT	1.03	0.82
*Bam*HI product	1.63	0.59

The four standard dinucleotides were prepared by phosphorylating the corresponding dinucleoside monophosphates (Collaborative Research) with polynucleotide kinase (Biolabs) and γ-^{32}P-ATP as described in the legend to Fig. 1 and purifying the products by electrophoresis on DEAE-cellulose paper in 7% formic acid (System 1). Oligonucleotides from the fingerprint shown in Fig. 1 were incubated in a reaction mix (20 μl) containing 66 mM glycine-NaOH pH 9.6, 6.6 mM $MgCl_2$, 3.3 mM dithiothireitol and 10 units exonuclease I for 30 min at 37 °C, and spotted on the appropriate paper for fractionation. The oligonucleotide labelled pGA in Fig. 1 was unaffected by this treatment and was identical with the products of digestion of all longer oligonucleotides. System 2 consisted of electrophoresis on Whatman 540 paper at pH 3.5 (ref. 8). R_B refers to the electrophoretic mobility with respect to that of the blue dye xylene cyanol FF[8] and R_Y to the yellow dye orange G[8].

The dinucleotide pGA was identified by (1) digestion with venom phosphodiesterase which liberated only pG; (2) its resistance to the action of exonuclease I[7]; and (3) direct comparison with the four standard dinucleotides pGN (Table 1). The sequences of longer oligonucleotides were inferred from their mobility shifts[9] and suggest a unique pentanucleotide pGATCC present at the 5′ end of each *Bam*HI digestion product. The presence of three of the four possible hexanucleotides pGATCCN indicates that specificity is lost at the sixth position—the absence of the fourth possible sequence being caused by the small number (six) of termini present in the digest used for this experiment. When λ DNA and $\Phi80suIII^+$ DNA were used in parallel experiments, all four possible hexanucleotides were observed (data not shown).

The simplest interpretation of these results is that *Bam*HI is recognising the hexanucleotide

$$\begin{array}{c} \phantom{5'\text{-}}\downarrow\phantom{\text{-A-T-C-C-3'}} \\ 5'\text{-G-G-A-T-C-C-3'} \\ 3'\text{-C-C-T-A-G-G-5'} \\ \phantom{3'\text{-C-C-T-A-}}\uparrow\phantom{\text{-G-5'}} \end{array}$$

with the sites of cleavage as indicated by the arrows. This would mean that *Bam*HI fragments possess a 5′-tetranucleotide extension and hence should be a substrate for a DNA polymerase. This prediction was tested by preparing *Bam*HI fragments of Ad-2 DNA and incubating them with avian myeloblastosis virus (AMV) reverse transcriptase in the presence of one α-^{32}P-deoxynucleoside triphosphate and three unlabelled deoxynucleoside triphosphates. All four possible combinations were used and the products were analysed by nearest-neighbour analysis. The results of these experiments are shown in Table 2

Table 2 Nearest neighbour analysis of nucleotides incorporated into *Bam*HI treated Ad-2 DNA by AMV reverse transcriptase

Labelled substrate	pmol ^{32}P-dNTP incorporated		Products of micrococcal nuclease and spleen phosphodiesterase digestion†				Sequence deduced (5′→3′)
	*Bam*HI treated Ad-2 DNA*	Ad-2 DNA*	Cp	Ap	Gp	Tp	
α-^{32}P-dATP	7.2 (6)	0.21 (0)	18	16	62	4	GpA
α-^{32}P-dCTP	6.8 (6)	0.10 (0)	13	12	10	65	TpC
α-^{32}P-dGTP	7.8 (6)	0.16 (0)	18	17	52	13	GpG
α-^{32}P-TTP	7.1 (6)	0.13 (0)	15	54	18	13	ApT

*Bam*HI fragments of Ad-2 DNA and untreated Ad-2 DNA were used as templates for the incorporation of one α-^{32}P-deoxynucleoside triphosphate and three unlabelled deoxynucleoside triphosphates with AMV reverse transcriptase. 23 μg (1 pmol Ad-2 DNA) of fragments were used in each experiment using conditions previously described[10]. An aliquot of the reaction mixture was precipitated with perchloric acid to determine the total incorporation. The resulting labelled DNA was completely digested with micrococcal nuclease and spleen phosphodiesterase[11] and the resulting mono-nucleoside 3′-phosphates analysed by electrophoresis on Whatman 540 paper at *p*H 3.5. The separated mononucleotides were visualised by radioautography, cut from the paper and quantitated by liquid scintillation counting.

*The number in parenthesis is the theoretical incorporation calculated from the deduced sequence and for the three *Bam*HI sites present in Ad-2 DNA.

†Quantities of 3′-mononucleotides expressed as a percentage of the total mononucleotides detected.

and provide confirmation of the recognition sequence assigned earlier on the basis of mobility shifts.

The data from these experiments involving repair synthesis indicate that there is some heterogeneity at the 3′ terminus in contrast to the homogeneity at the 5′ terminus. The amount of heterogeneity observed varied considerably from one experiment to another and is most easily interpreted as due to the presence of a contaminating 3′→5′ exonuclease (similar to exonuclease III of *Escherichia coli*)[12] present in the preparation of *Bam*HI used for these experiments.

Like *Eco*RI[13] and *Hind*III[14], *Bam*HI generates fragments bearing cohesive termini. This should prove useful for the production of recombinant DNAs and a vector suitable for *Bam*HI fragments has already been described[15].

Exonuclease I was a gift from R. Wu.

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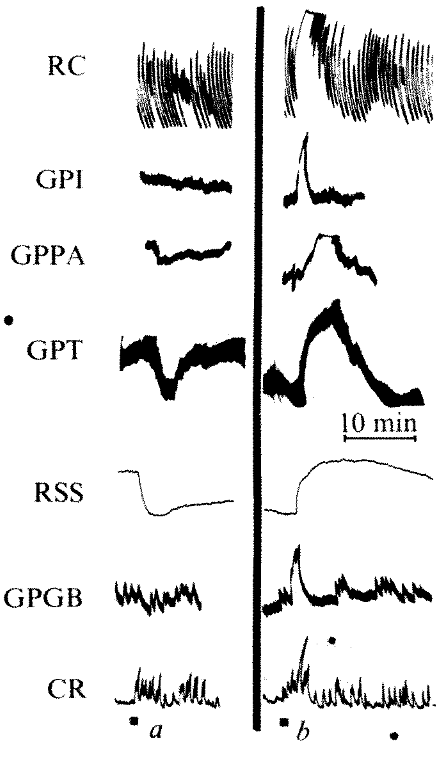
Relationship of spasmogenic and smooth muscle relaxant peptides from normal lung to other vasoactive compounds

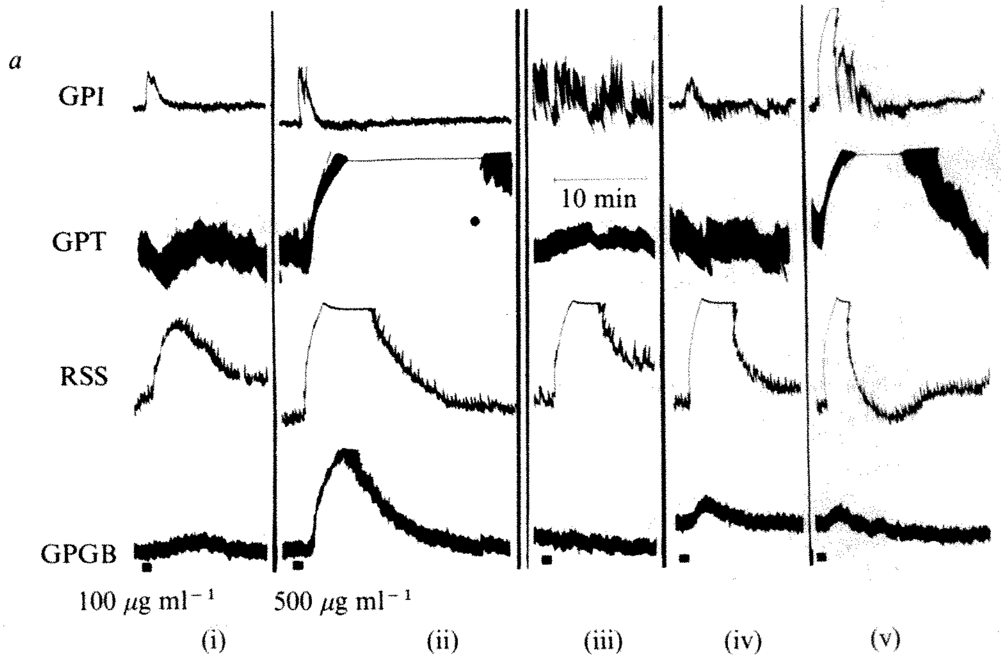
ALTHOUGH abnormal lung tissue, especially certain bronchogenic tumours, may secrete a variety of peptide hormones[1], none has been isolated from normal lung. We have now extracted from normal hog lungs peptide fractions containing at least two active principles, one of which relaxes and the other contracts non-vascular smooth-muscle organs. Like the recently isolated vasoactive intestinal peptide (VIP)[2], both pulmonary peptide fractions are systemic vasodilators. The relaxant peptide fraction further resembles VIP in its smooth-muscle effects, and binds with antibodies against this peptide. The spasmogenic peptide fraction contracts a variety of smooth-muscle organs that are also contracted by prostaglandins, bradykinin, angiotensin II, substance P, and the "slow-reacting substance of anaphylaxis". Immunoreactivity attributable to the relaxant, VIP-like peptide, was found in normal lungs of several mammals, including man, and in pulmonary oedema foam.

Fig. 1 Responses of isolated, superfused smooth-muscle organs to 125 μg ml^{-1} of *a*, relaxant and *b*, spasmogenic lung peptide fractions, infused at 1 ml min^{-1} (data from two experiments). RC, rat colon; GPI, guinea pig ileum; GPPA, guinea pig pulmonary artery; GPT, guinea pig trachea; RSS, rat stomach strip; GPGB, guinea pig gall bladder; CR, chick rectum. Assay organs had been pretreated with a mixture of pharmacological blockers containing: 10^{-7} g ml^{-1} mepyramine maleate, 2×10^{-7} g ml^{-1} methysergide bimaleate, 2×10^{-6} g ml^{-1} propranolol HCl, 10^{-7} g ml^{-1} phenoxybenzamine HCl, and 10^{-7} g ml^{-1} of hyoscine HBr. Peptide solutions were infused at mark below. Upward movement is contraction, and downward movement relaxation.



The extraction procedures were similar to those used in the isolation of VIP[3]. Fresh hog lungs were boiled for 10 min, minced and extracted in dilute acetic acid. The filtered extract was treated with alginic acid to adsorb peptides, which were later eluted with HCl, salted out with NaCl and extracted with methanol. During these procedures, repeated washing with lipid solvents ensured the removal of prostaglandins and other lipids. From the methanol extract, peptides were recovered in aqueous solution and then desalted and fractionated on a column of Sephadex G-25, with dilute acetic acid as eluant. The active fractions were chromatographed on a column of carboxymethyl cellulose (CMC), using phosphate buffer (*p*H 6.4) and a gradient of NaCl. Further purification of the smooth-muscle relaxant peptide fraction was obtained by a second chromatography on CMC, at *p*H 8, followed by counter-current distribution in a 1:1 mixture of 0.1 M ammonium bicarbonate and *n*-butanol.

During the extraction and purification, test solutions were assayed by their vasodilator action on the systemic circulation of anesthetised dogs[4], and by their effects on isolated, superfused smooth-muscle organs[5]. The active peptide fractions induced peripheral vasodilation, evidenced by an increase in femoral blood flow and a fall in aortic blood pressure on infusion into the femoral artery. When tested on smooth-muscle organs, these fractions were found to contain two active principles, having opposite actions. Thus, one fraction contracted, and the other relaxed: stomach strip and colon of rat; trachea, pulmonary artery, ileum and gall bladder of guinea pig; and chick rectum (Fig. 1). These effects were dose-related (Figs 2 and 3), were unaltered by pharmacological blockade of histamine, 5-hydroxytryptamine, adrenergic or cholinergic receptors, but were almost totally abolished by incubation with the proteolytic enzyme pronase (Calbiochem, 20 μg per mg peptide fraction).



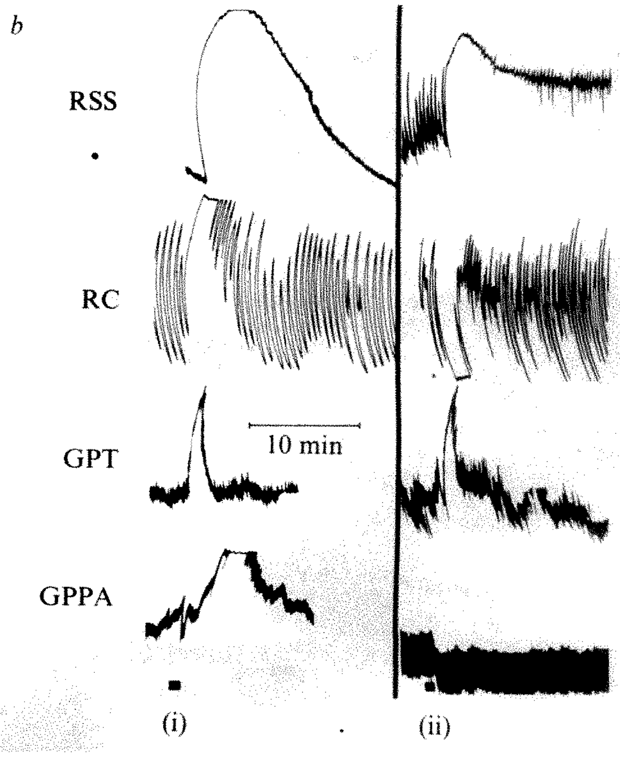


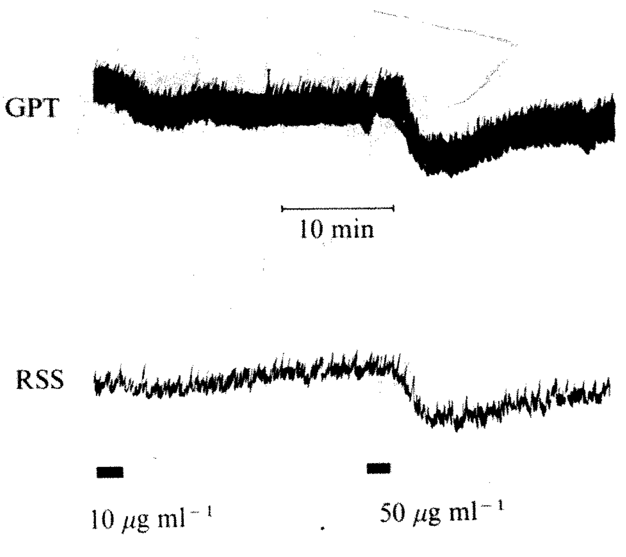
Fig. 2 *a*, Responses of guinea pig ileum (GPI), guinea pig tracea (GPT), rat stomach strip (RSS), and guinea pig gall bladder (GPGB) to two doses (100 and 500 μg ml^{-1}) of spasmogenic lung peptide fraction (i and ii) and to prostaglandin $F_{2\alpha}$ (50 ng ml^{-1}) (iii), prostaglandin E_2 (5 ng ml^{-1}) (iv) and substance P (1 μg ml^{-1}) (v). All agents were dissolved in Krebs solution and infused at 1 ml min^{-1}. *b*, Responses of RSS, rat colon (RC), GPT and guinea pig pulmonary artery (GPPA) to same spasmogenic peptide fraction (125 μg ml^{-1}) (i) and to bradykinin (50 ng ml^{-1}) (ii).

The smooth-muscle actions of the relaxant lung peptide fraction are similar to those of VIP, which relaxes the above-listed smooth muscle tissues[6,7]. This lung peptide fraction also competed with labelled VIP in binding to anti-VIP antibodies in a radioimmunoassy that detects 20 pg of the porcine peptide per ml[8,9]. With progressive purification, culminating in countercurrent distribution, this binding capacity increased along with the increase in biological activity and was at least 15% that of pure porcine VIP. The displacement of bound VIP with different dilutions of this peptide paralleled the standard curve for pure VIP. In the same assay, antibodies to VIP showed less than 1:1,000 cross reaction with secretin (GIH Laboratory, Karolinska Institute), glucagon (Eli Lilly), cholecystokinin–pancreozymin (GIH Laboratory), bradykinin (Sandoz), substance P (Beckman), or somatostatin (ovine, Beckman).

The spasmogenic peptide fraction, on the other hand, exhibited negligible ($<0.1\%$) binding capacity to VIP antibodies. The smooth-muscle actions of this peptide fraction resembled those of other vasoactive substances (Fig. 2), but could be distinguished from these by examination of the full spectrum of biological activity. Thus, prostaglandin E_2 in a dose (5 ng ml^{-1}) giving nearly equal contraction of guinea pig ileum, relaxed guinea pig trachea and gave a stronger contraction of rat stomach strip. Prostaglandin $F_{2\alpha}$ (50 ng ml^{-1}) contracted guinea pig trachea but was without effect on ileum and gall bladder. Substance P, in a dose (1 μg ml^{-1}) causing a stronger contraction of ileum, was less active on gall bladder and trachea. The spasmogenic lung peptide could also be distinguished from bradykinin, which relaxed rat colon and a strip of guinea pig pulmonary artery, and from angiotensin II, which is a vasoconstrictor. The incompletely identified "slow-reacting substance of anaphylaxis", was not available to us for comparison. This substance has been detected and assayed in biological solutions and tissue extracts on the basis of contraction of guinea pig ileum or trachea, in the presence of atropine and an antihistaminic[10]. These actions alone do not, however, permit a differentiation between the slow-reacting substance and the spasmogenic lung peptide.

Because of the above findings in purified peptide fractions from lung, crude extracts of normal lung from several species (dogs, cats, rabbits, and human subjects undergoing thoracic surgery) were examined for VIP-like immunoreactivity. The extracts, prepared by boiling for 10 min and extracted in acid alcohol, contained the equivalent of up to 2.8 ng immunoreactive VIP per g wet weight. On induction of pulmonary edema in anesthetised cats (by rapid intravenous infusion of dextran–saline solution) or in isolated, perfused cat lungs (by raising outflow pressure), oedema fluid and foam contained approximately 300 pg ml^{-1} of VIP immunoreactivity.

Fig. 3 Dose-related relaxation of guinea pig tracea (GPT) and rat stomach strip (RSS) in response to infusion of 10 and 50 μg ml^{-1} of relaxant peptide fraction.



The apparent presence in the lung of a VIP-like peptide may be explained by the embryological derivation of the lung from foregut, or by the presence of neuro-endocrine (APUD) cells, of neural crest origin, in the lung as well as the gastrointestinal tract[11]. This observation is another example of the occurrence of VIP-like bioactivity and immunoreactivity outside the gastrointestinal tract; related activity has recently been found in high concentrations in the brain and other nervous tissue[9]. The presence of two peptides with opposing actions on tracheobronchial and vascular smooth muscle (and other contractile elements in lung[12]) provides a potential mechanism for regulating the distribution of alveolar ventilation and perfusion. The demonstration of one of the vasoactive lung peptides in pulmonary oedema foam suggests that this, and possibly also the other peptide, may be released in clinical settings associated with pulmonary oedema, for example heart failure, "respiratory distress syndromes", oxygen poisoning, and anaphylaxis. The recent finding of increased plasma VIP immunoreactivity in some patients with lung tumours[8] could have been due to secretion of the VIP-like pulmonary peptide.

Because of the similarities in biological actions between the spasmogenic lung peptide, other vasoactive peptides (bradykinin, angiotensin II and substance P), the prostaglandins, and the "slow-reacting substance of anaphylaxis", the possibility of release of this peptide should be considered whenever spasmogens are released from the lung. The use of multiple bioassay organs should help to distinguish between the different compounds, especially if they are present singly.

This work was supported in part by a Lung Center Award from the National Heart and Lung Institute of the US Public Health Service, and by a grant from the Swedish Medical Research Council. We thank M. Rifaat, L. Melin, K. Thermaenius and T. V. Nguyen for assistance.

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Errata

In "Airborne ice nuclei near an active volcano" by R. C. Schnell and A. C. Delany (**264**, 535–6) the labels to curve families *c* and *d* in Figure 2 were interchanged. The upper family should have been labelled *d*, the lower *c*.

In "Suppression of murine lupus erythematosus by Dactinomycin" by A. E. Gabrielsen, A. S. Lubert and C. T. Olsen (**264**, 439–440) Figures 1 and 2 were interchanged—the captions are correctly placed.

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reviews

Historical essays on mycology

C. T. Ingold

Introduction to the History of Mycology. By G. C. Ainsworth. Pp. xi+359. (Cambridge University: Cambridge, London and New York, 1976.) £11.

MYCOLOGISTS have always lacked a comprehensive history of their subject. Many of the older mycologists have read with profit Whetzel's *An Outline of the History of Phytopathology* (1918) and have relished Ramsbottom's *The Expanding Knowledge of Mycology Since Linnaeus* (1941). And a much wider circle has also rejoiced in Large's *The Advance of the Fungi.* But now at last here is a full-scale history of mycology, although, with characteristic modesty, the author presents it as an *Introduction.*

Only one with an encyclopaedic knowledge of the literature on fungi could have produced this work which fittingly comes from the pen of one who can be described as the Dr Johnson of mycology. No other worker in this field can exist without almost daily reference to his *Dictionary of Fungi.*

Dr Ainsworth has sensibly not attempted to keep all sections of the subject in play while proceeding steadily from the earliest times to the present day, but has rather produced a series of historical essays on various aspects of mycology. These form his chapter headings: origin and status of fungi; form and structure; culture and nutrition; sexuality, cytology and genetics; pathogenicity; poisonous, hallucinogenic and allergenic fungi; classification; and finally the organisation of mycology.

Mayan mushroom stone (British Museum, London), probably from Guatemala and believed to date from the middle of the first millenium AD—early evidence for a mushroom cult in Central America.

P. A. Micheli, born in 1679 and the author of *Nova Plantarum Genera* (1729), is rightly regarded as starting the scientific study of the fungi; as the father of mycology. We learn from the English translation of his epitaph in Florence that he "was much beloved by all the worthy men of his age on account of his wisdom, sweetness of disposition and modesty". A number of figures from his magnum opus are reproduced: the full title-page; his lovely plate of *Clathrus;* his representation of agaric structure and development: his drawings of almost pure cultures of moulds on melon slices; his illustrations of *Aspergillus* and *Botrytis;* and his dynamic figure of a cup-fungus puffing. Also reproduced are Micheli's original drawings of asci, the first ever made, showing clearly that the blockmaker did a rather poor job at times in preparing the plates for the printed book.

There is a basic difficulty about a history that extends from the misty past to the present. In the earlier part much must be made from very little: and in the more recent part there is such a wealth of material that the historian is faced with an almost impossible task of selective condensation. It is, of course, easy to criticise this selection from recent history. For example, in connection with mycorrhiza no mention is made of Melin's work and the reader is referred for a recent view on ericaceous mycorrhiza to a 1927 review by Rayner! Again, in the genetics section the early work of Hansen and Smith in 1932 on heterokaryosis does not figure; indeed, the word does not occur in the index. But any criticisms of this nature are of marginal significance.

The chapter on pathogenicity is particularly rewarding because the author, with his profound knowledge of medical and veterinary mycology, presents a balanced picture in which diseases of plants and of animals (including man) are nicely balanced. Occasionally, perhaps inevitably, a chapter becomes something of a catalogue and this is most noticeable in the chapters on the distribution of fungi and on classification.

Illustrations are a special feature of the book. In all, 106 carefully selected figures—including reproductions of historic drawings; portraits and photographs of eminent mycologists; and facsimile reproductions of pages from books and manuscripts—provide a distinguished atmosphere for the book.

A brief section which many will enjoy deals with hallucinogenic fungi. The Wassons together with Roger Heim are regarded as the founders of ethnomycology.

The final chapter on the history of the organisation of the subject is stimulating. It is concerned with mycological societies, journals and books. Due credit is given to de Bary's textbook. Although over a hundred years old, this great work is still a model that has never been surpassed and is still an inspiration to working mycologists. The same chapter is enlivened by one of Worthington Smith's delightful cartoons of fungal forays a century ago.

Another mycologist writing a history (but it is difficult to think of anyone else who could do it) would certainly achieve a different balance. To some extent the balance reflects Dr Ainsworth's own interests but happily these are exceptionally wide. It is perhaps not a book for the undergraduate student, but it will be read with enormous pleasure by all mycologists with a broad interest in their subject. Every library in an institution where fungi are studied will have to possess a copy and many individual workers will want one of their own. ☐

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Insect integuments

The Insect Integument. Edited by H. R. Hepburn. Pp. xix+571. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl.165.00. $63.50.

THIS *Festschrift* is dedicated to A. Glenn Richards, author of the classic *Integument of Arthropods* (1951) and constructor of the first electron microscope ultramicrotome (1942). In a biographical first chapter, M. Rockstein describes the life and work of 'Glenn', including a bibliography. The miscellany of 26 papers naturally contains much that is already well-known. In the space here available I have therefore selected those items whose originality impressed me.

K. M. Rudall presents excellent X-ray diffraction diagrams from cuticles and from *Sagitta* (Chaetognatha) spines. Had one been more fully Miller-indexed, however, it would make the text easier to follow. V. Strout, H. Lipke and T. Geoghegan show by formic acid extraction a narrowing of the range of molecular weights of chitin as the last larval cuticle becomes that of the puparium in flies. This facilitates chitin reorientation at this stage.

In an invigorating chapter, R. A. A. Muzzarelli provides a useful collateral of chemical knowledge from fungal and bacterial research. This includes native chitosan, and the enzymes responsible for its formation from chitin and subsequent degradation. He also reviews chitin—carotenoid linkage in cuticles, and new insecticides acting as inhibitors of chitin synthesis, rightly warning of the danger of their indiscriminate use.

V. B. Wigglesworth emphasises the possible significance of lipids as structural polymers in cuticle. There follows a pioneer attempt (R. H. Hackman) to explain the large number of protein species in cuticles as polymorphism, due to substitution mutations in the second base of genetic code triplets. But the supporting evidence seems to require statistical analysis. S. O. Andersen provides a comprehensive review of cross-linking enzymes both in haemolymph and the cuticle itself. P. Karlson and C. E. Sekeris review the morphogenetic action of ecdysone, augmenting their classical results with recent evidence. P. J. S. Furneaux and A. L. Mackay describe a 90° cross-ply fibril array in a beetle eggshell, the first in insects, as far as I am aware. R. Dennell revives a mechanistic theory for contol of chitin orientation, but does not mention existing published evidence against it. H. R. Hepburn and I. Joffe have compiled useful and detailed tables of mechanical properties for chitin and whole cuticle. On the basis of stress–strain curves and fractography they distinguish cuticles which show plastic fracture, brittle fracture, or a combination of both. M. Locke relates cuticle formation to alternate secretion by plasma membrane plaques and Golgi vesicles.

S. Caveney presents results which may shed light on the unsolved problem of cell morphogenetic gradients. He shows that insect epidermis functions as a syncytium by cell/cell communication via gap junctions. The late J. M. Whitten records the regular orientation of centriole-like structures in the cells of fly feet, and suggests that they may be related to formation of regular hair patterns. E. P. Marks and B. A. Sowa are successfully exploiting *in vitro* cuticle growth methods for investigating blocks to chitin synthesis and effects of insecticides on cuticle formation under carefully controlled conditions. Chapters by H. Nemenz and W. Ebeling both stress the importance of epidermis rather than epicuticle as a water-controlling mechanism in relation to osmoregulation and insecticides respectively.

J. F. V. Vincent compares stress-softening in locust intersegmental membrane and filled rubber composites, while H. C. Bennet-Clark provides a lucid account of jumping principles and cuticular energy storage mechanisms. F. G. Barth summarises results of photoelastic analysis of strains applied to perspex models of the cuticular parts of arachnid mechanoreceptors. Ablation experiments have pinpointed the specific sense organs predicted to be responsible for the idiothetic sense of spiders. This is the sense of orientation using the experience of previous movement sequences, without using landmarks. H. E. Hinton reviews a wide range of recent work on insect physical colour systems. He records anti-reflection nipples (previously thought to be restricted to corneal surfaces) on the wing scales of the green hairstreak butterfly. W. Kenchington presents an intriguing range of modes of formation of peritrophic membranes. W. Peters summarises recent results on *in vitro* growth of peritrophic membrane, and factors which affect it.

The volume is well produced and will clearly be necessary reading for relevant workers—if their institutions can afford it. **A. C. Neville**

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Atmospheric radiative transfer

Radiative Processes in Meteorology and Climatology. (Developments in Atmospheric Science, Vol. 5.) By G. W. Paltridge. Pp. xvii+318. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl.103; $39.75.

THE basic atmospheric engine operates between a source which is derived from solar radiation absorbed by the atmosphere, or by the surface, and a sink which is the emission of infrared radiation to space. Radiation processes are, therefore, of fundamental importance to atmospheric study. Further, because of the current concern about the global climate and possible mechanisms through which climate may change, there is a lot of interest in the Earth's radiation budget and the possibility of small alterations which might occur in it, due to changes in the radiation input or output. For instance, variations in the net solar input might occur either due to changes in the sun itself or changes in the atmospheric cloud or dust content; or variations in the emitted radiation from the atmosphere itself might occur for similar reasons.

There are few textbooks available on atmospheric radiative transfer. One of the best of the standard texts written by R. M. Goody in 1964 is now out of print, so that this new text by Paltridge and Platt is very much to be welcomed. It presents the fundamental theory of radiative transfer, and shows how empirical and theoretical approximations may be applied to the transfer of radiation in the clear atmosphere and in clouds. A lot of very useful up-to-date information is contained in the book about the radiative properties of the surface, of different types of clouds and aerosol, and about atmospheric transmission in different spectral regions. Emphasis is placed on radiative processes in the troposphere; little is said about the high atmosphere.

The book does not set out to be a thorough theoretical treatise. In fact, some of the theoretical presentation is confused (for instance, the presentation of the Curtis–Godson approximation in chapter 7 and its confusion with the pressure scaling of absorber amount). Rather, the book is aimed at providing the methods and means for meteorologists and atmospheric physicists who need to incorporate radiation processes into atmospheric models or work out simple practical atmospheric problems involving radiative transfer. For such people the book will be very valuable indeed and well worth purchasing. **J. T. Houghton**

J. T. Houghton is Professor in the Department of Atmospheric Physics at the Clarendon Laboratory, University of Oxford, UK.

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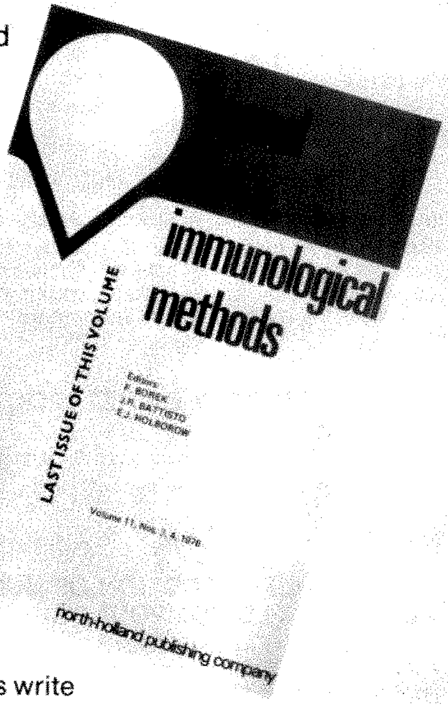
Studying items closely related to immunity such as complement, reticuloendothelial systems and others.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North American fossil vertebrates

Athlon: Essays on Palaeontology in Honour of Lovis Shano Russell. Edited by C. S. Churcher. Pp. 286. (Royal Ontario Museum: Toronto, 1976.) $15.

THIS *Festschrift* volume honours Dr Lovis Russell, who recently retired as Professor of Geology at Toronto University and Chief Biologist at the Royal Ontario Museum. Nearly all the 16 scientific papers are therefore concerned with aspects of North American vertebrate palaeontology, and over half of them discuss vertebrate morphology and systematics. These papers include the following: Dineley, on the mode of life of the heterostracan fish *Ctenaspis*, which seems to have ploughed through the surface layers of sediment; Olson and Lammers on a labyrinthodont amphibian, which seems to be intermediate between the trimerorhachoids and the brachyopoids; Morris, on the hypsilophodont dinosaur *Thescelosaurus*, prefers Thulborn's classification of the group to Galton's; Green and J. E. Martin revise the didelphid marsupial *Peratherium* of the North American Oligocene and Miocene; Turnbull reconstructs the jaw musculature of the South American sabre-toothed marsupial *Thylacosmilus* and shows that the stabbing action was by the action of the hyper-developed ventral cervical musculature, the relative proportions of the jaw-closing muscles not being of specialised carnivore type; Turner and L. D. Martin describe two new rhinocerotoids from the Oligocene of Nebraska; Hooijer compares the pygmy mammoth of the offshore Californian Channel Islands with other dwarf elephants; Lundelius and Slaughter revise the taxonomy of North American Pleistocene tapirs; and A. E. Wood revises the taxonomy of the Oligocene rodent family Ischyromyridae. Finally, at a somewhat higher phyletic level, Carroll redescribes the South African late Permian younginid genus *Heleosaurus*, which shows many similarities to the little early Triassic archosaur *Euparkeria*. He concludes that younginids may be ancestral to the archosaurs, and that the larger early Triassic archosaurs may represent an early divergent specialisation for a semi-aquatic habitat.

Five of the papers are faunal studies. Madeleine Fritz describes an unusual specimen made up of a series of overgrowths of two ectoproct species on a stromatoporoid base. Holman describes Cenezoic herpetofaunas from Saskatchewan and shows that, although the amphibian fauna of the early Oligocene was already essentially modern, the reptile fauna only became modernised between then and the late Miocene. Wann Langston suggests that the late Cretaceous Scabby Butte fauna was preserved in a marshier environment that lay closer to the sea than the more varied dinosaur faunas of the Edmonton and Oldman deposits. Storer shows that the Hand Hills Formation of Alberta contains both Mio-Pliocene mammals and Pleistocene mammals, the deposit resulting from glacial reworking and mixing. Finally, Hibbard shows that a stratigraphic confusion between three volcanic ash deposits in Kansas has led to the incorrect placing of the Cudahy Pleistocene fauna in the Yarmouthian instead of the Late Kansan.

In the most general (and therefore most generally interesting) paper, Sloan discusses the ecology of dinosaur extinction in western North America as shown by the replacement of the dinosaur-dominated *Triceratops* fauna by the mammal-dominated *Protungulatum–Stygimys* fauna that probably immigrated from Asia. He concludes that there is now good evidence of a major floral change at this time, there being a 45% drop in floral diversity and a 71% loss of plant taxa by extinction over 1–2 Myr. This change from a subtropical angiosperm-based flora to a cooler, temperate, more conifer-dominated flora was, Sloan suggests, due to climatic changes resulting from the retreat of the North American inland sea from the region.

Most of the papers are above-average in interest, the book is well produced and edited, and there are relatively few typographical errors. Even at prevailing rates of exchange, it is not expensive. As with any such collection, the main problem is that most workers will find that, however good the papers are, only a small proportion are relevant to their particular range of interests.

Barry Cox

Barry Cox is Professor of Zoology at King's College, University of London, UK.

Developmental biology

Explorations in Developmental Biology. By C. Fulton and A. O. Klein. Pp. xv+704. (Harvard University: Cambridge, Massachusetts, 1976.) £11.90.

THE authors of this book state that it is unsatisfactory to teach developmental biology through "formal presentation of a body of knowledge to be passively assimilated by students from books and lectures", and claim a novel approach in providing a collection of original papers, grouped in topics and linked by passages which clarify details, provide questions and suggest further reading. There are 59 papers, giving very broad coverage and dating from Spemann and Mangold (1924); but most of them are post-1960. Despite their praise of original papers and scorn for textbooks, the authors provide two text-type chapters (on amphibian embryology and cells in culture), abridge several papers and give an extract from Twitty's delightful *Of Scientists and Salamanders* rather than the original work.

Do any University teachers instruct in the way criticised by Fulton and Klein? The problem lies in balance. Since research papers are often difficult and time consuming to read critically, it is at least efficient to teach mainly by lectures and texts to elementary students, but to swing later to original sources, when the teacher's role becomes to select reading, help in discussion and provide factual background. The book duplicates this role and, of course, cannot keep up with the latest information as the teacher can.

A good textbook is not an *ex cathedra* statement of the truth. It stimulates by telling what is known, what is uncertain about what is known, what is unknown and how it might come to be known. As a summary of our knowledge, it can cover immense breadth, and at least mention the oddities. A comparison illustrates this. Fulton and Klein take 63 pages to discuss whether the statement that "genes do not change during embryonic development" is a "cornerstone of developmental biology" or a reasonable working hypothesis. Ebert and Sussex's *Interacting Systems in Development* covers the ground in 5 pages and asks more probing questions. So does Berrill's *Developmental Biology*, and adds the unusual case of *Ascaris*. Fulton and Klein make a hardly fair comparison with a brief and misleading paragraph in an introductory general biology text.

Would anyone find the book useful? Probably not those with access to an adequate library, teaching special aspects in tandem with a team of colleagues. For the teacher struggling to cover the field alone, it may prove a godsend. But a warning: in the areas I know best, the choice of papers and some of the linking commentary is idiosyncratic. This is of course the point about University teaching. It is done by individuals whose choice of emphasis provides an essential diversity. Fulton and Klein's course sounds fun, but it isn't mine. **J. R. Downie**

Dr Downie is a Lecturer in Zoology at the University of Glasgow, UK.

Metabolism in filamentous fungi

The Filamentous Fungi. Vol. 2: Biosynthesis and Metabolism. Edited by John E. Smith and David R. Berry. Pp. xiv+520. (Arnold: London, June 1976.) £19.75.

Much of the space allocated to this review could be taken up by listing the twenty-two authors and the seventeen chapters which they have contributed to this, the second volume of a series on the filamentous fungi. The writers endeavour to cover all aspects of biosynthesis of carbohydrates, lipids, fatty acids, nucleic acids and proteins and the associated pathways of intermediary metabolism and nutrient assimilation.

The authors, in their attempts to succeed in this, are forced to produce highly condensed articles some of which, although they may be comprehensive, are very hard work to read. Many authors, however, do set out to make their contributions readable and comprehensible to those not working directly in their own field. A. T. Bull and M. E. Bushell begin by providing a good introduction to the problems encountered in the construction and operation of continuous flow fermenter systems for filamentous fungi.

A. H. Rose provides an excellent account of the clinical nature of membrane components. At the other end of the spectrum, one or two authors make few concessions to anyone who is not fully conversant with the terminology and techniques used in their discipline, and consequently have produced chapters which are very difficult to digest.

The latter is not consistent with the editors' intent to include undergraduate and postgraduate students in the readership. Although perhaps not all subjects would have been amenable to this treatment, it would have been useful if the editors had encouraged authors to begin their chapters with a brief introduction to the nomenclature. For example, the chapter on Carotenoids by T. W. Goodwin begins with a good introduction which rapidly accelerates to a detailed exposition with which the reader can keep in step.

An encouraging feature of this book is that a number of chapters conclude with suggestions for useful future developments, whereas volumes presenting up-to-date reviews often leave one with the feeling that most worthwhile work has been done or is in hand. V. W. Cochrane, in the closing paragraphs of his chapter on glycolysis, encourages workers in the field to investigate *Neurospora crassa* in the same detail with which bacteriologists have studied *Escherichia coli* and also points out that some groups of fungi have been largely neglected. In another instance, J. A. Pateman and J. R. Kinghorn have attempted to present their review of nitrogen metabolism in such a way as "to allow deeper study for those interested in a particular field".

This is a valuable volume as it provides a good review of present knowledge, with copious references, and it may well have the effect of encouraging others to investigate some of the neglected areas of the subject.

H. A. S. Epton

Dr Epton is a Lecturer in Botany at the University of Manchester, UK.

Numerate account of marine ecology

The Ecology of the Seas. Edited by D. H. Cushing and J. J. Walsh. Pp.x+467. (Blackwell: London and Oxford, 1976.) £8.75.

The names of Cushing, Dugdale, Kelley, Parsons, Steele and Walsh, to mention but 6 of the 14 contributors, should be sufficient to promise good value in this book for marine ecologists. I have no doubt that it is good value, and to be highly recommended.

What these very numerate ecologists aimed to do, under the leadership of Cushing and Walsh, was to set out "the present state of knowledge of the quantitative nature of marine ecosystems": in 6 parts, comprising 16 chapters, with very adequate references and indexes (authors, subjects, species and geographical). They proceed systematically from the characteristics of the seas and the life they maintain, through the forms and functions of marine ecosystems, with some of the consequences in terms of evolution, and of yield for man. The final section is in two parts: the first, an excellent chapter on sampling (by J. C. Kelley), and the second concerning a comprehensive "simulation model" of ecological relationships at three trophic levels, within the upwelling systems off Peru, Baya California and north-west Africa.

In all this, prime emphasis is on the processes concerned, both hydrodynamic and biological: indeed, life *in* the sea. In association there is emphasis on the absolute necessity for distinguishing between variability in space and variability in time, which variations have been "confounded" in more senses than one for most aspiring marine ecologists over the past 50 years.

I cannot but be impressed by the way in which so many of the problems —which could be seen only dimly 50 years ago and, although perhaps perceived a little more clearly 25 years ago, still left marine ecologists gravelled for lack of both data and technique—are now (following great developments in both) beginning to yield understanding, and even the possibility of prediction, in such complicated circumstances. That the methodology is far from simple, and the options many, is further illustrated by a quotation from the last chapter, by Walsh (references omitted): "Depending on the problem, one must decide whether steady state or time dependent studies . . . with simulation and/or statistical models . . . involving the analytical or numerical solution . . . of differential or difference equations . . . made of linear or non-linear terms . . . should be solved on analog or digital computers . . . in relation to spatial or single point considerations . . . and exogenous or endogenous driving functions . . . within a stochastic or deterministic parameter space . . .".

As Cushing says in his preface, the contributors are writing as scientists, who have spent long periods at sea, for scientists who are beginning to work at sea. I find it difficult therefore, to think of a better or more challenging introduction. Whether this important science will, however, be able to expand in foreseeable circumstances, in the manner that progress made so far seems to justify, is uncertain to say the least. Quite apart from never-ending arguments about the Law of the Sea, it is inherently very costly, if only in terms of ships and resources required for the extensive synoptic surveys that better understanding demands. For this reason alone, one of the most important points made by Walsh concerns the present challenge to devise new methods of exploiting existing data banks, rather than heedlessly adding to them!

One last point: taking into account the high price and unattractive binding, it is difficult to understand why the publishers let the team get away with duplicating Fig. 7.6 by the identical (except for the legend) Fig. 8.2.

C. E. Lucas

Sir Cyril Lucas was formerly Director of the Marine Laboratory, Aberdeen (retired 1970) and subsequently a member of the Natural Environment Research Council, UK.

announcements

Awards

Alfred Champagnat (France) is the winner of the 1976 Unesco Science Prize, and **Louis Estrada Martinez** (Mexico) and **José Reis** (Brazil) will share the 1974 Kalinga Prize for the popularisation of science.

A prize of DM 10.000 is donated by Boehringer, Mannheim, every two years for outstanding work in the field of biochemical instrumentation and analysis. One or several papers concerning one theme, either published or accepted for publication between October 1, 1975, and September 30, 1977 should be sent to: Prof. Dr I. Trautschold, Medizinische Hochschule Hannover, 3000 Hannover 61, Karl-Wiechert-Allee 9.

The Science Research Council intends to award about fifteen fellowships in 1977 under its new **Advanced Fellowship Scheme.** These fellowships fill the gap between SRC postdoctoral and senior fellowships schemes. They will be awarded to outstanding young workers who have already proved their ability but have not yet obtained a tenured post. The Fellows would have no guarantee of a tenured post on completion of their fellowship although most would be expected to gain such posts in due course. The stipend will be linked to the University lecturer scale. Details of the regulations are available from the Science Research Council, State House, High Holborn, London, WC1. Applications for advanced fellowships to commence on October 1, 1977 should be sent to the Science Research Council by January 31, 1977.

Appointments

Members of the **Genetic Manipulation Advisory Group** have been announced by the Secretary of State for Education and Science. They are Sir Gordon Wolstenholme (Chairman), J. B. Brooksby, K R Dumbell, H. J. Evans, J A. Gilbey, R. J. C. Harris, M. Jahoda, Bernard Langley, J. E Lawrie, John Maddox, K. Mather, J. Ravetz, M H Richmond, J. H. Subak-Sharpe, Sir Brian Windeyer. Further appointments are expected shortly.

Michael John Bevis, Reader in Metallurgy and Materials Science at Liverpool University, has been appointed to the newly created Chair in Non-Metallic Materials at Brunel University. Professor Bevis will become Head of the Department formerly called Polymer Science and Technology.

F. John Bryant of the Department of Physics, Hull, has been appointed to a Personal Professorship.

Person to Person

The possibility exists of arranging postdoctoral research in investigations of the kinetics of chemical reactions relevant to terrestrial and planetary environments. Topics currently under study include the kinetics of the formation, decomposition, and photodissociation of mesospheric cluster ions, and the mechanisms of production of interstellar molecules. Informal enquiries should be addressed to R. R. Burke, CRPE, CNET & CNRS, 45045 Orléans Cedex, France.

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Reports and Publications
Outside UK—October

US Department of Commerce: National Oceanic and Atmospheric Administration. Marine Geology and Geophysics Data Services and Publications. Pp. 11. (Boulder, Colorado· National Geophysical and Solar-Terrestrial Data Center, 1976) [2110

Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Division of Irrigation Research, 1974–75 Pp. iv + 79. (Griffith, NSW· CSIRO, 1976) [2110

Bulletin of the American Museum of Natural History. Vol 157, Article 4 Shell Ultrastructure of the Atlantidae (Heteropoda, Mesogastropoda) *Oxygyrus* and *Protatlanta*, with Comments on *Atlanta inclinata*. By Roger L. Batten and Michael P. Dumont. Pp. 263–310 (New York American Museum of Natural History, 1976.) $2 90. [2210

United States Department of the Interior: Geological Survey. Professional Paper 869 Noncystimorph Colonial Rugose Corals of the Onesquethaw and Lower Cazenovia Stages (Lower and Middle Devonian) in New York and Adjacent Areas By William A. Oliver, Jr. Pp. v + 156 + 108 plates Professional Paper 882. Recent Surface Movements in the Baldwin Hills, Los Angeles County, California By Robert O Castle and Robert F Yerkes. Pp. vii + 125 + 4 plates. (Washington, DC. US Government Printing Office, 1976) [2210

Smithsonian Contributions to Zoology. No. 230. Contribution to the Polychaete Family Trochochaetidae Pettibone. By Marian H. Pettibone Pp 21. No. 231· Benthic Marine Cypridinacea from Hawaii (Ostracoda). By Louis S. Kornicker. Pp iii + 24. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [2510

Chemical Evolution Comparative Planetology. (The Third College Park Colloquium on Chemical Evolution, September 29–October 1, 1976) Pp 57. (College Park, Maryland Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland, 1976) [2510

The Phaistos Disc An Interpretation of Astronomical Symbols By Leon Pomerance. Pp 76. (Goteborg Paul Astroms Forlag, 1976) [2510

CERN—European Organization for Nuclear Research. CERN 76–15. Electronic Equipment for Wire-Stretching Machines. By N. Rasmussen Pp. vii + 15. (Geneva. CERN, 1976.) [2510

United States Department of the Interior. Geological Survey. Professional Paper 929. ERTS-1: a New Window on Our Planet. Edited by Richard S. Williams, Jr., and William D. Carter Pp. xix + 362. (Washington, DC US Government Printing Office, 1976) [2510

United States Department of the Interior Geological Survey. Water-Supply Paper 2131 Surface Water Supply of the United States, 1966–70. Part II· Pacific Slope Basins in California. Vol. 4· Northern Central Valley Basins Pp. ix + 747 (Washington, DC US Government Printing Office, 1976) [2610

Reactor Centrum Nederland. Summary of Activities July 1975–July 1976 Pp 20. ('s-Gravenhage· Reactor Centrum Nederland, 1976) [2710

Ozone Depletion in the Atmosphere. an Annotated List of References. (Library Bibliography Series No. 3) Pp. 24. (Orlando, Florida: Florida Technological University, 1976.) [2710

Eine neue Theorie uber die Gravitation aus Kybernetischer Sicht Von Johan C. Metz Pp. 26. (Aix-la-Chapelle. Johan C. Metz, Huttenstr. 66, 1976) [2710

Annals of the Transvaal Museum. Vol. 30, No 7 The Tenebrionidae of Southern Africa, XLIV A New *Gonopus* (*Agonopus*) Species from the Transvaal (Coleoptera) By L. Schulze. Pp. 91–95 + plate 9. Vol. 30, No. 8. On a Newly Associated Composite Mandible from Swartkrans (Mammalia· Hominidae). By T. D. White Pp. 97–98 + plates 10 and 11 Vol 30 No. 9: Trap Response of some Southern African Small Mammals. By J. A J. Nel and I L' Rautenbach. Pp. 99–104. Vol. 30, No 10. The Type-Material of the Nominal Species *Charaxes rausoni* Van Someren (Lepidoptera Nymphalidae) By R. I. Vane-Wright Pp 105–107. (Pretoria Transvaal Museum, 1976) [2710

Diagnostic Kinetic Energy and Potential Vorticity Calculations for Monthly Mean Atmospheric Flow. By Hannu Savijärvi. (Report No. 9) Pp 11. (Helsinki. Department of Meteorology, University of Helsinki, 1976.) [2710

The Periodic Table Based on Electron Configuration. (Beau-Bassin, Mauritius Raymond Rivet, 1976.) £1.20; $2 20. [2710

CERN—European Organization for Nuclear Research CERN 76–16. Construction of a Prototype Superconducting Quadrupole Magnet for a High-Luminosity Insertion at the CERN Intersecting Storage Rings. By J Billan, R Perin, L. Resegotti, T. Tortschanoff and R. Wolf. Pp 32. (Geneva. CERN, 1976.) [2810

World Reputation Trends· Signs of Hope, Signs of Stress. By Lester R. Brown. (Worldwatch Paper 8.) Pp 400 (Washington, DC. Worldwatch Institute, 1776 Massachusetts Avenue, N. W., 1976) $2. [2910

Canada Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 239. The Alkaline Rocks of Canada By K. L Currie. Pp. 228 (Ottawa. Information Canada, 1976) $8. [111

Massachusetts Institute of Technology Lincoln Laboratory. Seismic Discrimination. Semiannual Technical Summary Report to the Defense Advanced Research Projects Agency, 1 January–30 June 1976. Pp viii + 104 (Lexington, Mass. Lincoln Laboratory Massachusetts Institute of Technology, 1976) [111

Netherlands Organization for the Advancement of Pure Research (Z.W O) Current Research in the Netherlands: Mathematics, Physics, Geology, Astronomy, 1975. Pp 244. (The Hague Netherlands Organization for the Advancement of Pure Research, 1976.) [211

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. There is a Reader Enquiry Form on the page facing the inside back cover.

Automated X-ray powder diffractometer. Philips. The APD 10 comprises a Philips 3 kW generator with vertical goniometer equipped with graphite monochromator and 35-sample changer, plus associated controls and chart recorder; the additional automation facilities are controlled by a minicomputer using plain language instructions *via* a teletypewriter. A dual station cassette unit enables measurement data to be collected for off-line processing on a larger computer. Standard programs are available. The APD 10 enables routine work to be done overnight or at weekends. Applications cover geology, mining, steel production, petro-chemicals, paint and air pollution.
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Double beam atomic absorption spectrophotometer. Pye Unicam Ltd. The SP2900 has both peak height and peak area measurement which can be remotely initiated. The timing for peak height measurement is from 0–99.9 seconds in 100 msecond steps and peak area integration is over a 4 or 10 second period. The built-in lamp turret keeps four pre-focussed lamps aligned, powered and ready to work. Lamp selection is optical. The SP2900 has aircraft-style, 7-bar, bi-polar illuminated digits. The range is −0.05 to 1.999A with a switchable decimal point and double over-range indication. Readout is in absorbance, concentration or emission units and digital update is 2.5× every second. A concentration calculator helps produce fast and accurate results with a single operation of curvator and scale expansion controls using just two standard solutions. The single curvature control is independent, wide-ranging and does not affect zero. Calibrated scale expansion is from 0.2× to 50× (analogue and digital). The background correction accessory provides automatic, simultaneous correction for high background absorbances—particularly important when using electro-thermal atomisation techniques. The SP2900 corrects for 1.5A of background to better than 1%.
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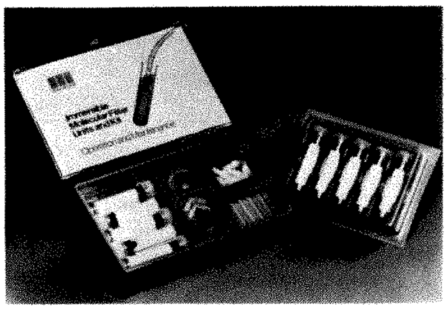
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Multi-point recording facility. Chandos. A new electronic attachment provides multi-point recording facilities from a single channel recorder. Two or more of these units could be used with existing dual channel or multi-channel recorders if they are continuous trace. The system provides a channel identification datum and a means of adjusting the time spent recording individual points. Models accommodating up to 36 points are available for various types of measurement—pressure, temperature, pH, conductivity, oxygen, selective ions etc. Adjustment and zero suppression can be built in to provide a given full scale deflection.
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Miniature accelerometer. SE Labs. The EGAL 125-5, measures acceleration, vibration and shock from 0.001g to 5g. Weighing less than 0.5 g and occupying less than 0.006 cubic inches, the EGAL is ideal for situations requiring minimal weight addition to the item under test. Applications vary from tremor studies on human and animal subjects to vibration and flutter analysis on vehicle models undergoing wind tunnel tests. The EGAL is a semiconductor strain gauge device which measures static and dynamic acceleration with a sensitivity of 15mV/g unamplified. Available with six different mounting facilities, it has an operational temperature range of −40 to 121 °C. In addition, it is available with a 0.7 critical damping option.
Circle No. 49 on Reader Enquiry Form

nature

January 13, 1977

RADIOPHYSICS
C.U.

Catch 22

We don't often comment editorially on the stories of Soviet policy towards dissidents that we carry with monotonous regularity. This is not because we don't believe them—it's because they have a peculiar eloquence of their own to which cosy editorialising from a London office can add little. Sufficient it is that scientists should be aware on a worldwide basis of what goes on and act accordingly. The same goes for the testimony of Vladimir Bukovskii on the following page. But one story emerging from Moscow so confounded our sense of logic that we feel it deserves a little comment.

Academician Veniamin Levich was briefly taken into custody on December 21 as part of a new campaign against participation in the unofficial scientific seminars for dismissed scientists. He was referred to a TASS statement of November 9 concerning the Soviet position on emigration. Since this provoked no overseas reactions, he was told, this must imply tacit agreement.

The unattributed statement, one of many issued on or around Revolution Day, was broadcast in English at 07.36 and ran, in part, "each request from an individual citizen to be allowed to go and live in another country in order to reunite split families is given full consideration. Refusals are made in an insignificant number of cases, when the citizen expressing the wish to leave the USSR has had access in the course of his duties to works or information that represent a state secret . . ."

Since "the secrets of a state are always its own exclusive property", it went on, "it is an internal question for the Soviet Union to decide which specific works or information are to be considered secret, obsolete, or to have lost importance in guaranteeing the defences and other important national interests of the USSR". Accordingly, "any attempt at pressuring competent Soviet organisations into changing their position is considered an interference in the domestic affairs of the USSR. As for the big anti-Soviet noise raised in the West, it pursues the sole aim of misleading public opinion . . . Bourgeois propaganda is doing everything it can to distract attention from the systematic and gross violations of basic human rights in capitalist society".

In view of the strong hints that a big anti-Soviet noise is an interference in the domestic affairs of the USSR it is somewhat remarkable that the said "competent Soviet authorities" now quote the lack of "noise raised in the West" as a justification for their policy. ☐

No export potential in steam tape-recorders

"Parlez-vous electric?", runs a recent large advertisement by the UK Electricity Council in national newspapers, at a cost of goodness-knows-how-many-thousand pounds. "There are thousands of tape-recorders with headsets in use in UK schools. Thousands more are used each year by executives of multi-national companies on six-week intensive courses in a foreign language. Headsets. Video tape-recording equipment. Magnetic tape. All these modern educational aids are powered by electricity. The child learns his language idiomatically. So do the men. Men responsible for earning this country millions of pounds in export orders from foreign countries. Exports are this country's life blood. Electricity helps generate them. **Think Electric**".

"Look here, Blenkinsop," said the Minister for Energy, the moment the Director of the Electric Power Generating Board had sat down. Blenkinsop knew immediately that this was to be the dreaded eyeball-to-eyeball confrontation. He fumbled for his pipe. "I'm getting fed up with the way everyone else in the Cabinet is talking about export, export all the time—boasting about their departments' achievements—and we're still importing in our department. I get a lot of stick for it, so you'd better get your advertising copywriters to do something about brushing up our image. There must be something we export. Oh, and also", Blenkinsop shifted uneasily, "I've been looking at electric consumption figures for these last few weeks. What's going on? We're 5% down on last year, and I've got all these boilermakers, miners, nuclear engineers and what-have-you on my back about building more power stations. I want you to get out and stimulate consumption in a really big way. Find something which really soaks up electricity and push it hard." "Yes, sir" said Blenkinsop. The minister pulled a drawer open. Blenkinsop wondered if it was to be a letter asking for his resignation. But no; the minister decanted three or four small boxes on the table.

"Know what these are?" "No, sir". "Really, Blenkinsop, you're not very alert to the opposition. They are tape recorders—but they don't run on electricity. This is a clockwork one—it punches a tiny paper tape. It's made in Switzerland by all those watchmakers put out of business by digital watches. It sounds a bit like a 78-record, I admit, but you see how they'll develop it. This one's made in France. It runs on lighter-fuel, and I hardly need remind you that North Sea oil doesn't yield lighter-fuel so it'll have to be imported. And this one is made by our own dear old Gas Board; that's a shot across your bows, isn't it, Blenkinsop? Now you get people cracking; they've three big campaigns to do on export, consumption and competition".

"Yes, sir", said Blenkinsop, as his civil-servant brain swung into action "but, sir, if I can save us money with some sort of integrated campaign, do you thing I might, er . . . er . . . be so bold as to have a Permanent Secretary's 10′ 9″ × 7′ 3″ thick-pile carpet for my office? I only really qualify for the Deputy Secretary's 8′ 3″ × 6′ 4″ rug".

"Oh, very well" said the minister, irritably, and out trooped a gleeful Blenkinsop. "Odd people, these civil servants," mused the minister, "I wonder what he meant by an integrated approach . . . I hope he doesn't make a fool of us". ☐

The Bukovskii perspective

Vladimir Bukovskii, the dissident Soviet biologist released after years in and out of prison and psychiatric hospitals, answers questions from Vera Rich

VR : Do you think that your work in science, studying biology, in any way led you to the protest movement?

VB : I began to take part in the protest movement before I became a student. But my interest in the biological sciences and my studies helped. Science in the USSR (I don't mean the 'social sciences') has followed a path from being completely ideological to being partially so. It is said that at one time mathematicians were forced to prove the class nature of the integral. I did not belong to that period. In my time the physical sciences were already treated with great honour because of their defence potential.

Biology was far less lucky in this respect. Even in 1960, when I entered university, the father of Soviet biology was still Lysenko; genetics and biocybernetics were only just beginning to defend their right to exist. The very methodology of higher education; the abundance of unnecessary Party discipline; the barrack-like spirit; the dependence and the lack of rights of the students, who were blackmailed into joining the Komsomol and informing on their comrades by the threat of losing their grant and expulsion from the university . . . When I, at last, was expelled for "failure to conform with the character of a Soviet student" (for taking part in youth meetings on Mayakovskii Square), I was simply glad, to tell the truth. But my interest in biology remained, and later, even in prison, I continued my self-education as far as possible.

Under the influence of biology and while I was still at school my first disagreement with the ruling doctrine arose. The cornerstone of Marxism, the slogan "Being determines consciousness", evoked active protest in me. Society appeared to me as a living organism, and social consciousness, being the sum of individual consciousness, was the determinant of social processes. This incompatibility gave a stimulus to the analysis, criticism, and rejection of Marxism. Later the very fact of working with science, which develops in a person a careful concern with the truth, a discipline in thought and a training in patience, completed this process. It is not by chance that later there was wide participation of mathematicians, physicists and biologists in our movement. The scientific approach to legal questions, which was established in the first place by mathematician Aleksand Vol'pin, exerted an enormous influence on the whole development of our movement.

VR : How do you see your own case in the context of the aims of the Sakharov Human Rights Committee?

VB : I consider that the activity of the Sakharov Human Rights Committee in Moscow is a continuation of the process of scientific consideration of the problems of rights and injustices in the Soviet Union about which I have just been speaking. This process, in my opinion, belongs to the man of the future if he, like Andrei Dmitrievich Sakharov, will conscientiously, magnanimously and readily unite to defend all those who are being prosecuted. Like all my comrades in prison, I have always known that Sakharov remembers us, is concerned for us, and defends all of us. In the Vladimir prison we came to know that Sakharov had enumerated dozens of names of political prisoners in his Nobel lecture. For me personally it is clear that the activity of Sakharov and his Human Rights Committee was one of the origins of the wide international campaign for my release.

VR : Taking into account the existence of psychiatric persecution, what do you think is the proper position for Western psychiatrists? Should they boycott all contacts with the Soviet Union? Should they continue to take part in conferences in the Soviet Union, in the hope of exerting unofficial pressure on Soviet psychiatrists? Should the Soviet psychiatric profession be excluded from participation in international conferences until there is a change of policy towards dissidents?

VB : I consider the generalisation "the Soviet psychiatric profession" is not justified in all respects. There are different schools, currents and types of psychiatrists. It is almost impossible to speak of some single policy adopted by all psychiatrists towards dissidents.

In the last examination at the Serbskii Institute in autumn 1971, when, under public pressure, the authorities included among the experts several honest scientists such as Professor Melekhov and Professor Lukomskii, I had no questions regarding the profession's policy towards 'dissidents'. I did not doubt for a second that their decision would be objective.

In 1965 when, on the orders of the KGB, I was taken by force to City Psychiatric Hospital No. 13 in Moscow, young and completely unknown psychiatrists did not fear to declare me to be sane. Even in psychiatric prisons I met a few decent psychiatrists. Decency in these conditions amounts to heroism. Are you asking me whether I want to exclude these people from the world psychiatric profession, to break off their scientific contacts with you, or yours with them?

On the other hand, is it right to sit down at the same table with Lunts and Snezhevskii, hoping to exert 'unofficial pressure' on them? Their mere presence at the same table as Western psychiatrists gives them respectability, introduces them into the ranks of psychiatrists and makes you accessories to their crimes. By no pressure can you return to them their long-lost consciences. The very idea of behind-the-scenes deals with murderers and executioners is incompatible with the conscience of a true scientist. The 'diplomatisation' of psychiatry has already led to a number of cowardly compromises: contacts between western psychiatrists and odious figures of Soviet psychiatry, and the refusal to discuss openly and to condemn Soviet psychiatric malpractices. It must be understood that the Soviet authorities consider such behaviour to be proof of the West's weakness which only strengthens the repression. It is also very convenient for those in the West who are prepared to have contacts with the Soviet Union at any price.

Is it not remarkable, then, that people educated in the traditions of Western democracy, and accustomed to expressing their opinions vigorously and to considering honour in their relationships with each other, should think it is possible and necessary to conspire, scheme and even compromise their consciences, provided contacts with the Soviet Union are involved? Yet it is not only tactically more correct, humanly speaking it is easier to maintain the direct honourable position of a scientist. One does not have to force one's conscience. No one ever has to sit down at the same table with those known to take part in psychiatric repression. In any case it is necessary in the course of discussions and resolutions at any international meeting to speak openly to Soviet psychiatrists, saying all you think about the repression, and thus to extend the hand of help to the honest scientists who stand bashfully behind their dishonest colleagues. It is necessary to expand the participation of Western psychiatrists in the struggle for each victim of psychiatric repression. Only in this way, without and within, can one help cure Soviet psychiatry and establish in it such people as my comrade in prison, Semeon Gluzman. □

No season to be jolly

David Spurgeon reports from Ottawa on recent nuclear developments in Canada

CANADA'S nuclear programme has been in something of a turmoil lately. Certainly for officials of Atomic Energy of Canada Ltd. (AECL), the Crown corporation charged with nuclear R&D, exports and technical aid, the season has definitely not been jolly—quite the reverse. The opponents of nuclear power have stepped up their attacks in public, in AECL's eyes sometimes quite unfairly. The auditor-general has questioned their accounts. And that has led the House of Commons' Public Accounts Committee to investigate what looked like highly unusual and extraordinarily large payments to agents in such unlikely places as Panama and Liechtenstein; these had apparently been made as commissions on CANDU reactor sales to countries like Korea and Argentina, which many thought should never have been sold reactors at all.

The auditor-general's report then added insult to injury by revealing that AECL lost $100 million on one of these deals, though it was later disclosed that this loss could be reduced to $25 million by yet another sale now being negotiated with the same country, Argentina. It was shortly after all this appeared in front-page newspaper headlines that the Minister of External Affairs, Mr Donald Jamieson, announced a new nuclear export policy which because of its stringent requirements for safeguards will probably restrict overseas sales of reactors in the future.

This was less a direct result of the foregoing events, however, than a product of India's detonation of a nuclear device in May, 1973, with the help of a Canadian research reactor. But it has already had a paradoxical effect: since Pakistan would not agree to the terms of the new policy, Canada's nuclear aid programme with that country will be cut off, in spite of the earlier contention of the Prime Minster, Mr Trudeau, that Canada should not withhold nuclear technology from developing countries. Mr Jamieson has admitted to the press that there is a real possibility that the nuclear policy decision will cause electrical brownouts in Karachi.

To some people, all this seems to strengthen the suggestion made not long ago by John Shepherd, executive director of the Science Council of Canada, that AECL should give up its export sales programme of CANDU reactors and concentrate on exploiting what could be a huge domestic market. To do this properly, he proposed that AECL should revert to its R&D function and help form a consortium with private nuclear industry and the provincial power companies.

Long and complicated

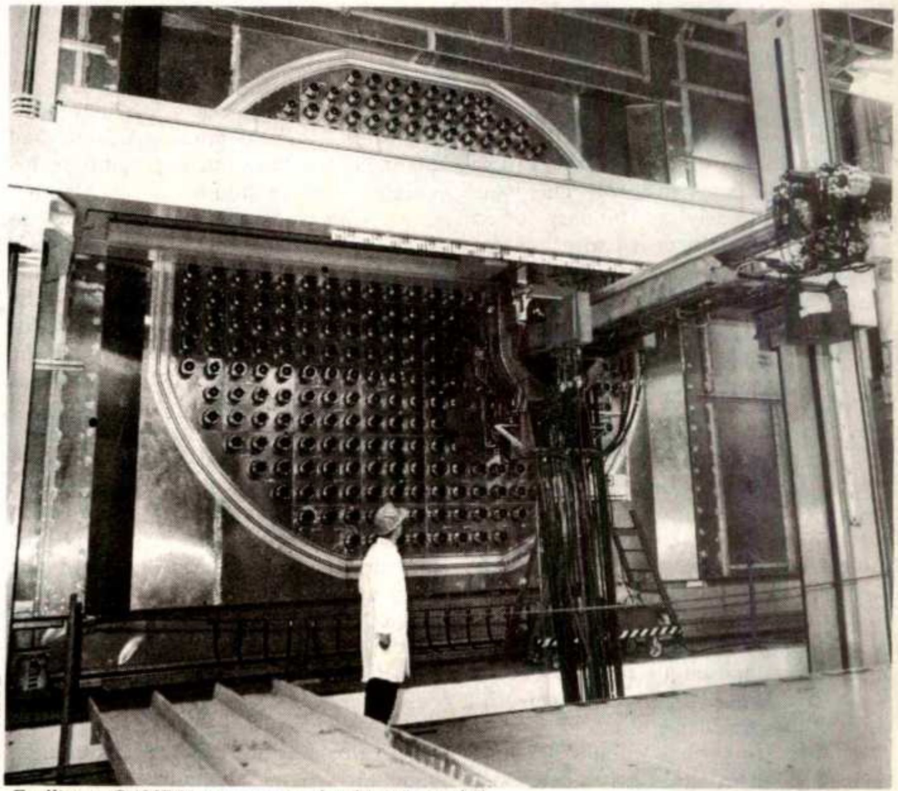
The story of the commissions on CANDU sales is a long and complicated one not yet revealed in its entirety to the Canadian public. One commission, on the Korean sale, amounted to $18.5 million. The company involved, United Development Inc. (UDI), reported that it paid $1.3 million of this to the Triangular Trading Company of Panama as a portion of the $8.1 million it claimed for expenses in the deal.

UDI, whose headquarters are also listed as being in Panama, also paid fees totalling more than $1 million to firms in Hong Kong and Frankfurt for technical and economic studies of CANDU compared with other nuclear systems. AECL agreed to pay the fees after it had renegotiated the agent's contract and reduced the commission from about 5% to 1%, plus documented expenses.

UDI is headed by Shaul Eisenberg of Tel Aviv, and it was the inadequate documentation for the $8.1 million expense account paid to him that prompted the auditor-general, J. J. Macdonell, to report it to parliament. Mr Macdonell made it clear that AECL officials tried to persuade him not to make the matter public, and AECL's chairman, Ross Campbell, admitted this but said he was concerned not about the facts but about the innuendos that would damage AECL's reputation and those of the companies it dealt with. In fact, a special study of AECL's financial management on behalf of the auditor-general was strongly critical of its overall handling of expenditures. This study, unrelated to the Korean sale, was part of a continuing review of Crown corporation's management.

The second commission case involved a joint sale by AECL and Italimpianti of a CANDU reactor to Argentina. The Italian firm obtained an agent for this sale and then asked the Canadian government to pay half the cost. Canada responded by having $2.4 million deposited into a Swiss bank account held by a trading firm in Liechtenstein, as requested, but never learned the name of the agent or whether it was the Liechtenstein firm.

The Italian firm stalled on providing the answer, moving the Canadian Energy Minister, Alastair Gillespie, to wonder publicly if "there may be Canadians involved." He said the Royal Canadian Mounted Police had been asked to find out who received the $2.4 million, if they could.



AECL

Fuelling a CANDU reactor: no shutdown is needed

Exports restricted

The new nuclear policy announced by Mr Jamieson just before Christmas means that Canada will restrict its exports of nuclear equipment and materials to countries that either have signed and ratified the Nuclear Non-Proliferation Treaty or have "full-scope" nuclear safeguards. In addition, these countries will have to accept the kinds of additional bilateral safeguards announced by Canada two years ago. The alternative to adherence to the NPT means that a country must accept equivalent international safeguards on its complete nuclear programme (outside military aspects if it has nuclear weapons already). This is what Pakistan refused to do.

Announcing the policy at a press conference, Mr Jamieson claimed that Canada now has the toughest requirements imposed by any nation supplying nuclear materials. He said Canada decided on the policy only after it had failed to persuade other members of the so-called London Club to take similar steps together. Canada now hopes that these other nuclear-supplying countries will follow its lead, which is designed to prevent the proliferation of nuclear weapons. And he suggested that recent tightening of policies by the United States and France was a hopeful sign.

Canada's new policy is seen also as a reinforcement of a safeguards policy announced two years ago, which some uranium customers have not been observing closely. A number of countries, including Japan, Switzerland and the EEC countries, have not yet completed all the required safeguards. After allowing two six-month extensions of supplies, the Canadian government has announced that it will cease uranium shipments at the end of December, 1977, to force compliance with the policy. Mr Jamieson said he hoped that by that time the safeguards would be in place and supplies could continue uninterrupted.

In notes distributed before his press conference, Mr Jamieson said:

> Nuclear export policy already requires binding assurances that what Canada provides will not be used for explosive purposes. Existing policy, however, does not cover what a country receives from other suppliers or what it might do on its own. The new policy will close this gap. We will have, therefore, assurance by treaty that Canada's nuclear customers will have been selected from amongst those countries which have made a clear and unequivocal commitment to the non-proliferation of nuclear weapons.

Mr Jamieson added that Canada recognises the legitimate energy requirements of its trading partners, but is determined to do all it can to avoid contributing to nuclear weapons proliferation. It was for that reason that Canada had unilaterally decided to strengthen further her safeguards requirements. As in the past, "We are prepared to accept the commercial consequences of being clearly ahead of other suppliers. This is the price we are prepared to pay to curb the threat to mankind of nuclear proliferation." □

Canadian High Commission

Don Jamieson, External Affairs Minister

Holland's nuclear problems

Casper Schuuring reviews a troubled year for nuclear power in the Netherlands

IF it had not been so before, nuclear energy became a major political topic in the Netherlands in 1976. The issue was alive as early as January when debates on the subject led to a government decision to postpone definite planning for three 1,000 MW nuclear power plants until after the next elections in May 1977. The Netherlands already has two existing smaller plants, of 447 MW and 54 MW.

Overcapacity in generating power and uncertainties regarding the nuclear fuel cycle were among the main reasons for the move, but political factors were important too. The small Radical Party (PPR), which takes part in the country's coalition government with Labour and Christian Democrats, has two ministers and an under-minister very much opposed to nuclear energy —one of whom is Fokele Trip, the Minister for Science Policy.

Labour, too, has doubts about nuclear power. The Christian Democrats are less perplexed. One of their ministers, for Economic Affairs, had planned the three nuclear plants in his energy memorandum of September 1974. Construction of the first was to start in 1976. Following the government decision to postpone the building of the plants, a declaration signed by 1,200 scientists covering a range of disciplines expressed their concern at the rapid expansion proposed for nuclear energy—as 2,300 colleagues of theirs had done earlier in the United States. Published at the end of January and originally addressed to the government, the declaration was then directed to parliament, which was to discuss the memorandum in February. With the decision on additional nuclear plants pushed to the next government, however, the parliamentary discussions lacked excitement.

Calm doesn't last

The calm was not to last. A conflict soon arose over a credit guarantee to be given by the government for the building and selling of components for two power plants in South Africa. The work was to be done by a group of industries participating in a consortium together with American and Swiss industries. The government had already indicated in mid-1975 that an export licence would be given, but in parliament a vote of censure was just passed covering exports of nuclear material and equipment to countries that had not signed the nuclear Non-Proliferation Treaty. South Africa is such a country.

The consortium was originally to have built two reactor vessels; but with a change in arrangements the vessels were to be built by General Electric and the Dutch industries could still take part in the work, for which only an export guarantee was then necessary. Discussion went on so long that at the end of May the deadline passed and a French consortium won the South African order. The Dutch government itself just survived a long and critical period, and relations between government and industry sustained a good deal of damage.

During the summer the Steering Group for Energy Research issued its second report. This concluded that conservation of energy should form a considerable part of Dutch policy, and offered two scenarios for energy use over the next 25 years. One involved a continuing growth of 4½% until the year 2000, and the other a levelling off to zero growth around 1995. The latter scenario, which the Group itself backed, still involved building 6,500 MW of nuclear plant in 2000; the government and electricity producers were planning between 10,000 and 14,000 MW at that time (Holland now has a total generating capacity of 14,000 MW and plans 39,000–56,000 MW by 2000). The nuclear research centre was meanwhile transformed into a general energy research institute, incorporating work on wind, fusion and flywheel programmes; a national programme on solar research is foreshadowed for early this year.

Breeder intention

By last autumn the Economic Affairs Minister Mr Lubbers was informing parliament almost casually of the intention of provincial and local electricity producers to take part in Superphénix, France's first commercial fast breeder. Some time before, the minister had shown some antipathy towards continued Dutch cooperation with Belgium and Germany—the three countries are building the 300 MW SNR–1 in Kalkar and are each carrying out a part of the research.

In a letter to parliament at the end of August the minister confirmed that the Netherlands was in principle continuing with the Kalkar project until SNR–1 was finished, and that Belgium and Germany had agreed to make a decision on a commercial breeder (SNR–2) then. He added that the electricity producers were involved in the SNR project, and were also in the larger European group which will develop the breeder concept further. This group, he said, would proceed with the building of the 1,240 MW Superphénix, which is based on the Phénix, before the SNR–2; as a decision to build this would be taken in the beginning of October, the electricity producers had to decide on their role.

An urgent debate was held in parliament, mainly because the Radical and Labour parties thought the Netherlands would become involved in the commercial development of a breeder before some problems were solved and vital experience was gained. As a result of one of the votes in parliament, Dutch participation is only acceptable once very strict conditions are made regarding safety aspects and international control of the project. The electricity producers agreed, and accepted the maximum financial contribution imposed on the 4,000-million guilders project, though not without some disagreement amongst themselves. By the end of the year, however, no decision to build the breeder had been taken.

Fourth time

When another nuclear subject attracted attention in October, it was for the fourth time in the year, and in this case created a problem lasting until close to Christmas. The problem concerned uranium enrichment using the gas centrifuge technique, and a definite solution is not actually expected before the end of February. The Anglo-Dutch-German group known as Urenco has pilot plants in Capenhurst in Britain and Almelo in Holland, and partly finished demonstration plants. The difficulty is that a decision is due on extensions to increase Almelo's capacity to 1,200 tonnes. Towards 1987 a further expansion for Urenco to 6,000–8,000 tonnes is foreseen, and the consortium has definite orders for 2,000 tonnes enriched uranium a year from 1983.

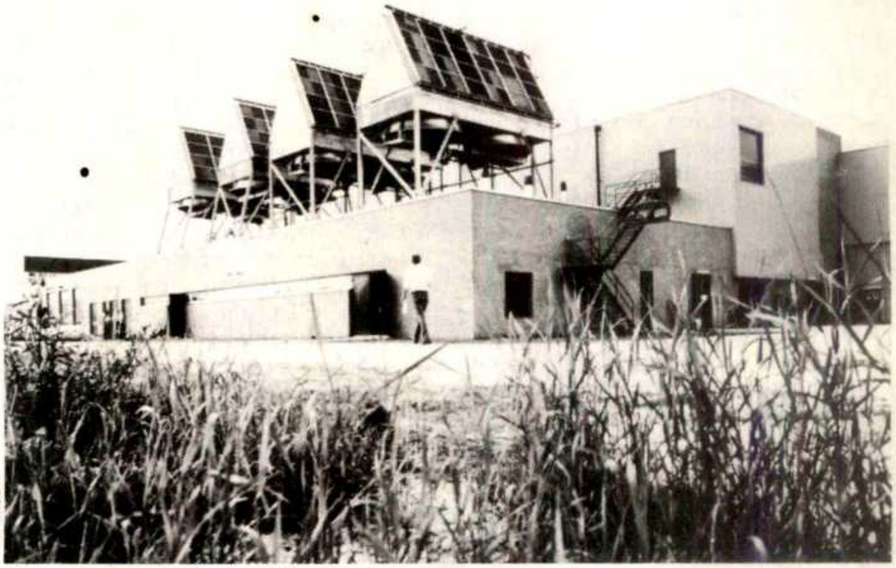
The Radicals are firmly against an expansion of the enrichment capacity for reasons connected with proliferation. There are also commercial objections, and these come not only from the PPR. Mr Lubbers has given confidential information to the parliamentary commissions for foreign, economic and nuclear affairs, but there is hesitation and doubts remain over both proliferation and the possibility of a costly overcapacity in the international enrichment market. The German–Brazilian deal is seen as particularly worrying. The Reflection Group on Energy Policy—active in 1975 before the decision on the three power plants was made—wrote a memorandum on the issue, and the PPR's own group of consultants published a report.

At the same time as all this happened the announcements from the USA and France of restrictions on nuclear exports became known, as did details of the resistance in Germany to a rapid expansion in the number of nuclear power plants. A further important point was that large commercial concerns like Shell, Philips and DSM, originally involved in the project, did not like to invest more money in it for both economic and political reasons. They believe it is impossible to work when political aspects play such a great role in decision-making.

State of crisis

A state of crisis existed for several weeks in The Hague, but the cabinet finally decided to agree provisionally to participate in Urenco's expansion, which also involves the Capenhurst plant. The parliamentary committees were informed, but it is still not clear what precisely the Dutch ministers of economic and foreign affairs will discuss with their British and German colleagues on the matter. The decision could mean domestic political problems. The Radicals are still against the idea and could conceivably leave the government. Labour and the Christian Democrats, however, are not willing to export enriched uranium to countries which have not signed the NPT. Each member of the tripartite consortium has the right of veto according to the Treaty of Almelo of 1970, and in Holland the tendency now seems to be to have this exercised not only over the export of uranium but on all nuclear exports.

At the very least, whether this government breaks up over the issue or not, the nuclear power debate will prove important in the formation of the next government. Developments during 1976 have seen to that. ☐



Urenco

Urenco's plant at Almelo, Holland

Judgment of the people

Colin Norman reports from Cambridge, Massachusetts, on the recommendations of a citizens committee established last year to look at genetic manipulation experiments

In a report likely to have important and widespread implications, a committee of citizens of Cambridge, Massachusetts, has recommended that experiments involving a new technique for manipulating genes in living organisms—recombinant DNA experiments—should be allowed to go ahead at Harvard and Massachusetts Institute of Technology. The committee said, however, that the experiments should be permitted only if they are placed under safety controls more strict than those now in force in most other institutions, and that a special board, consisting of Cambridge residents who have no affiliations with the two universities, should be established to monitor recombinant DNA research in the city.

The committee was set up last summer by the Cambridge City Council after a shrill and bitter dispute had erupted over plans to conduct recombinant DNA experiments at Harvard and MIT. At the urging of the city's colourful and irascible Mayor, Alfred E. Vellucci, the council also placed a three-month moratorium (later extended until January 7) on some types of recombinant DNA research in Cambridge while the committee conducted its investigation.

The report is far from being the last word on the matter, however. Even before the document had been formally presented to the council at a meeting held on Janaury 5, Vellucci told a few reporters that he disagreed with the recommendation that the research should be allowed to proceed in the city, and he said that he will propose a resolution at a later council meeting that some types of recombinant DNA experiments be outlawed entirely in Cambridge. Though it is generally considered unlikely that Vellucci's view will prevail in the council, the shouting is clearly far from finished.

The committee's work and its final report have won qualified praise from both sides in the dispute and, if nothing else, it has provided ample support for the view that citizens groups are well qualified to consider and pass judgment on complex technical issues. The committee, which was chaired by Dan Hayes, a former Cambridge Mayor, was thrust into the middle of a highly charged political and scientific conflict, and it has acquitted itself admirably.

Events in Cambridge are important in the context of national and international efforts to control recombinant DNA research. Those efforts began in 1974 when a group of scientists went public with their concerns that genetically modified microorganisms produced in recombinant DNA experiments might pose a hazard to public health if they should escape from the laboratory and survive in the environment. Last June, the National Institutes of Health (NIH) published a set of guidelines designed to guard against such a possibility. They define four levels of physical containment to be used for recombinant DNA experiments, designated P1 to P4, and the guidelines assign experiments to them on the basis of potential risk.

In addition, the guidelines specify that some experiments should only be carried out on crippled strains of microorganisms which have a limited capacity to survive outside the laboratory. The restrictions formally apply only to research supported by NIH, however, and they have encountered considerable criticism from opponents of the research.

While the guidelines were being drafted, plans were drawn up to construct a safety laboratory at Harvard at which some recombinant DNA research would be undertaken. Under the definition in the NIH guidelines, it would be a P3-level facility. A similar laboratory had already been constructed at MIT for cancer research, and it was also expected to house some recombinant DNA experiments. The proposed Harvard lab became a source of dispute between opponents and proponents of the research, and the conflict spilled over into the city council after an article was published in a local newspaper.

Town-gown relationships have never been very friendly in Cambridge, and Vellucci quickly latched on to the issue. After two extraordinary council meetings in late June and early July at which the warring scientific factions presented their views, the Council imposed a moratorium on P3- and P4-level experiments in Cambridge and asked the citizens' committee to recommend the circumstances, if any, under which such research should be permitted.

The committee's investigation is thus the first detailed review of the NIH guidelines to be carried out by a group composed entirely of nonscientists. It should be emphasised, however, that it was asked to consider only P3 and P4 experiments, and thus it was not asked to consider the rationale used by NIH in assigning experiments to different safety levels. It was also asked to consider only the potential health hazards of the research and it therefore steered clear of such emotive issues as whether the research might lead to undesirable applications.

After conducting lengthy public hearings and poring through reams of written material, the committee reached the unanimous conclusion that P3-level experiments can be permitted in Cambridge under certain conditions. Since no P4 facility is planned in the city, the committee said nothing about P4-level experiments, though it implied that they should not be carried out there. The chief condition set by the committee is that all recombinant DNA experiments should be performed with strict adherence to the NIH guidelines, but it also recommended the following additional safeguards:

- All laboratory personnel should receive adequate training in safety procedures, and institutions planning to carry out such experiments should produce a manual of procedures.
- Under the NIH guidelines, biohazards committees must be established in institutions where recombinant DNA experiments are conducted, and the Cambridge committee recommends that they should contain technicians and at least one representative from outside the institution.
- All P3 level experiments conducted in Cambridge should use crippled strains of microorganisms.
- Institutions must conduct tests to ensure that experimental strains of organisms are pure and that they are not resistant to common antibiotics.
- Institutions in which recombinant DNA experiments are performed must "in good faith make every attempt, subect to the limitation of the available technology" to monitor for the escape of host organisms, and "this should include whatever means is available to monitor the intestinal flora of the laboratory worker".
- Finally, the committee's most interesting recommendation is that a Cambridge Biohazards Committee (CBC) be established "for the purpose of overseeing all recombinant DNA research that is conducted in the City of Cambridge". The Board would be composed of people appointed by the city manager and its members would have no affiliations with the universities. Its functions would include reviewing pro-

posals for recombinant DNA research to ensure that they would comply with the NIH guidelines, maintaining links with the biohazards committees at Harvard and MIT, and "developing a procedure for members of institutions where the research is carried out to report to the CBC violations either in technique or established policy".

In addition, the committee urged Congress to pass legislation extending the NIH guidelines to cover all recombinant DNA research in the United States and to set up a national registry of laboratory workers for long term epidemiological studies.

The report has been welcomed by supporters of the research. David Baltimore, a tumour virologist at MIT's Cancer Research Center, said in an interview last week that the report "is basically a vindication of the whole process which began with the NAS committee", which raised the first concerns about the potential hazards associated with the research.

Opponents of the research are disappointed that the committee didn't come down in favour of banning the research in Cambridge, but they also agree with much of the report. In particular, Jonathan King, an MIT biologist who has been among the more outspoken opponents, noted in an interview that creation of the CBC would be a good step toward more citizen control over research and it would lead to increased accountability of scientists to the public. He also welcomed the suggestion that the health of laboratory workers be monitored, but suggested that unless pressed the universities would drag their feet in implementing the recommendation.

The ball is now back in the City Council's court. At the meeting on January 5 when the report was publicly unveiled, the council passed a resolution extending the moratorium on P3-level experiments in Cambridge for a further 30 days to allow time to consider the recommendations and to hear responses from the universities. Vellucci has promised to introduce a resolution banning P3- and P4-level experiments entirely and making scientists who conduct such research liable for a fine of $1000 per day. Another resolution will be proposed to implement the committee's report.

- One Cambridge Councillor, David Clem, said last week that he expects the report to be well received, and that the matter will probably be cleared up before the extended moratorium is scheduled to end. He noted, however, that he has some reservations about the recommendations and will wait to see what plans the universities have for implementing them before he decides how to vote.

Finally, it should be noted that other local governments, particularly the New York Attorney General's office, are taking a close look at the control of recombinant DNA research. The fact that a committee of non-scientists has recommended in favour of going ahead with the research, albeit under strict controls, will not pass unnoticed. □

PAKISTAN

New policy on the anvil

Azim Kidwai reports from Karachi on changes expected in Pakistan's science policy framework

THE economic, social and cultural changes that have occurred under the Bhutto government—they include nationalisation of almost all domestic banks and insurance companies, of the heavy industrial and parts of the agricultural sector, but not consumer goods industries like textiles, tobacco, chemicals and pharmaceuticals—have naturally had an impact on science policy. A new national policy now on the anvil is the result of lengthy deliberations amongst the elite of the scientific community and representatives of various scientific organisations and government agencies. Final proposals are expected once they have been discussed at Cabinet level.

The existing arrangements for scientific and technological research in Pakistan appear to suffer from three weaknesses: coordination of effort at national level is lacking; the universities have a very weak base as far as scientific research goes; and no effective mechanism exists for the utilisation of the results of research. At the apex of the structure of science in Pakistan lies the Ministry of Science & Technology of the central government assisted by the National Science Council and the Pakistan Science Foundation.

The National Science Council was created as a coordinating body for the different Research Councils and to advise the government on promotion of science in general, but in practice rarely discharged those functions effectively, possibly because it had no financial or budgetary strings to pull. It thus remained but a shadow of what it was originally conceived to be. The Pakistan Science Foundation was created subsequently as a sponsoring body to help promote financial support for university research work and promising individual research workers, for pilot plant studies, for the establishment of science museums and for scientific societies. This it has been doing within the limits imposed by the funds at its disposal.

This triangle at the top lacked coordination, and in practice was not up to implementing a vigorous science policy fully integrated into the national development effort. Suitably informed decisions could not be made at the top political level, and administrative coordination was inadequate, particularly in respect of planning. The proposals for the new science policy try to take account of these weaknesses. A National Council for Science and Technology is proposed as the supreme body to provide guidance on all matters pertaining to formulation and implementation of government policy on science and technology. It should also be responsible for planning, coordination and cooperative implementation of scientific and technological programmes. The proposed National Council may be headed by the Prime Minister, and the central ministry for science is to act as its secretariat.

The Research Councils—the Pakistan Council of Scientific and Industrial Research, Pakistan Medical Research Council, Irrigation, Drainage and Flood Control Research Council, Council for Works and Housing Research, Agricultural Research Council and the recently approved Livestock Research Council—are likely to be given autonomous character and resources to organise mission-oriented research in their specialised fields.

Some idea of the scope of R&D effort through these institutions can be gained from the budget figures for the current year, which reflect to some extent their relative weaknesses and strength. These are shown in the accompanying table. □

Pakistan's science research budget 1976–77

Council	Rupees
Scientific & Technological Research Division of the Ministry	1,998,000
National Science Council	443,000
Pakistan Science Foundation	5,500,000
Pakistan Council of Scientific and Industrial Research	29,320,000
Pakistan Medical Research Council	1,506,000
Irrigation, Drainage and Flood Control Council	740,000
Council for Works and Housing Research	820,000

£1 = 16.5 Pakistani rupees

correspondence

The nuclear debate

SIR,—I believe *Nature* is right to enlarge the nuclear debate to the general, international scene but I wonder if both sides can be represented fairly. Döderlein's essay (November 18, page 202), which presents one side of the issue, needs to be balanced by these points:

- The battle is one-sided. Pro-nuclear propagandists are paid; the opposition is not. Most nuclear "experts" are employed by the nuclear industry, which has money to spend. For example, anti-nuclear forces were outspent four to eight or more times in the six states where initiatives were balloted in November (*Science*, November 19). Another disquieting factor is that nuclear industry representatives seem to want to exclude biologists, environmentalists and other concerned members of the general public from the controversy. This approach does not generate confidence.
- Your opponent is always "irrational", as I find Döderlein. I also object to his suggested dichotomy: "reasonable, rational and valid" (or "reasoned, factual and rational") versus "irrational, emotional and ethical". One wonders what Döderlein's definition of rational might be.
- In my opinion humanity should be glad certain persons " . . . play out some of their inner and emotional needs by taking an active part in the nuclear controversy . . . ". These "needs" may be a safety valve for society. Humans resist becoming robots, they have emotions and let us hope they will continue to learn to express them for our collective betterment. Also, "emotional" and "irrational should not be confused; an emotional approach can be disciplined by rationality and even by the scientific method. Emotionlessness is not unjustly linked with death.
- The fact that already " . . . industry . . . gives uncontrolled releases . . . of substances which remain toxic for infinite times . . . ", if true, would hardly be an argument for accepting nuclear wastes. Furthermore, all toxic materials do not have the same toxicity and we are not obliged to make an unweighted choice among them.
- Practically all the plutonium present on earth, contrary to Döderlein's implication, has been produced by man-made nuclear reactors. No natural source of plutonium is known although trace quantities have been reported in uranium deposits. Any plutonium that might have existed on the primitive earth (of 4.6×10^9 years ago) would have long since disappeared because of plutonium's relatively short half life.
- "Medical" reactors may be much smaller than the bomb-capacity commercial reactors planned, hence medical reactors and small research reactors are less directly linked to the spread of nuclear weapons. I believe many people, some nuclear experts included, would like to see *more* nuclear research. We are alarmed by the planned installation of hundreds of big reactors all over the earth without enough research having been done, especially on medical and genetic risks to nuclear workers and the general public. Some of these reactors will contain *tonnes* of plutonium (see Frank Barnaby, The SIPRI Yearbook, 1976). Inhaling a few microgrammes can kill you.
- Finally, Döderlein asks " . . . can anything short of actual war stop a determined sovereign nation from getting primitive but usable versions of any kind of contemporary weapons technology—nuclear, bacterial, chemical or any other?" I say war could lead to have-not nations getting nuclear and other modern killing techniques, especially if such countries align themselves with the "right" side in the conflict. In this context, both sides would probably be the right side. So, let us try something short of war.

It seems to me that instead of trying to restrict the discussion to experts we should indeed extend it to everyone. This would encourage a human synthesis, an overall approach on world problems that no one discipline or industry can provide. Our capacities for arriving at common solutions will obviously have to be developed. This could be helped by de-specialising scientific and philosophical methodologies, which could be combined and applied to questions such as disarmament, the energy and food crisis and nuclearisation of the earth. A convergence and interaction between science and philosophy is necessary for just solutions and a stable future world, if it is to be democratic.

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CERN'S project management

SIR,—In your article of November 25 marking the completion of the CERN 400 GeV SPS you paid rather less attention than might have been expected to the fact that the project has not only comfortably exceeded its original specification in several important respects, but has also been completed on time and within its budget. The excellence of CERN's project management is self-evident, but the matter cannot be left there.

It has been said that CERN is an exacting customer both technically and financially, because it takes great pains to define its requirements precisely and because it usually has a clear idea in advance of how much it will need to pay. On the other hand, it can be a cooperative if tough customer, providing technological expertise, advice and highly-qualified personnel to contractors who experience problems during production. The advantages to both sides in this close relationship are obvious, even though the efficient use of outside technological help may sometimes have to be imposed upon an unwilling firm.

Building the SPS has involved the development of working relations with hundreds of firms throughout Europe. Although CERN's contributions to advanced technology and the economic utility of its contracts have been fully documented[1,2], its influence as a good customer has perhaps been underestimated, if only because the immediate financial gains are almost impossible to quantify. Now that the customer-contractor principle is established as a fundamental concept behind much British research funding, it is essential to re-emphasise the point that the ultimate success of a complex scientific project depends as much upon the competence and scientific expertise of the customer as upon the performance of the contractor.

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[1] Meeting on Technology arising from High Energy Physics, Proceedings, CERN 75–9 (1974).

[2] Schmied, H., A Study of Economic Utility Resulting from CERN Contracts, CERN 75–5 (1975).

The Editor of *Nature* welcomes all correspondence. Letters should be kept as brief as possible.

news and views

Nations and numbers 1976

from Robert M. May

LAST year I gave a summary (*Nature*, **261**, 12; 1976) of the 1975 estimates of population magnitudes, growth rates, age structures, and patterns of urbanisation which were compiled for individual countries and for regional groupings by the Environmental Fund (1302 Eighteenth St, Washington, DC). Their 1976 wall-chart shows much the same figures, with the total world population swollen by about 94 million to an estimated 4,241 million, and still growing at around 2.2% per annum.

The many uncertainties in population estimates are perhaps best illustrated by the figures for the USA. The Environmental Fund gives the mid-1976 population to be 222.2 million, as opposed to the US Bureau of the Census figure of 215.7 million. The discrepancy arises because the official figure does not include the undercount of about 5.3 million which has been announced by the Bureau for its most recent census in 1970, nor does it include an estimated 1.2 million illegal immigrants (constituted of 800,000 who entered illegally and evaded deportation, and 400,000 from among the 11 million foreign visitors who entered legally during the past year).

Such uncertainties are writ large for China, which has issued no official figures since 1956. Estimates of the current Chinese birth rate range from 37 to 17 per 1,000. The Environmental Fund chart uses Aird's 'intermediate model' (Foreign Demographic Analysis Division, US Department of Commerce, 1975) for the population of China. This is substantially higher than the US Bureau of the Census estimate, whose world population figure is consequently 4,069 million. The UN estimate for China is yet lower; highest is the CIA estimate.

Overall, the UN estimates the world birth rate to be 33 per 1,000, one less than it was 20 years ago. The Environmental Fund chart shows 35 per 1,000. As available data are not accurate to better than 2 per 1,000, the exact number is anyone's guess.

The chart also summarises the number of acres of arable land per person (from the 1974 United Nations Food and Agriculture Organisation, *Production Yearbook*, **28**). Although there are considerable difficulties in definitions here, the trends are interesting: an average of 1.2 arable acres per person in Africa; 0.5 in Asia; 2.3 in North America; 0.8 in Latin America; 0.7 in Europe (0.3 in the United Kingdom); 2.2 in the USSR; 5.3 in Oceania. Among countries with more than 10 million inhabitants, low is 0.1 for Holland, Japan and South Korea, and high is 8.1 for Australia.

Of the 158 countries listed in the chart, 35 were net exporters of cereal grain in 1948–1952; 18 remained in 1974 (UNFAO *Trade Yearbook*, **14**, 1960 and **28**, 1974). By regional grouping, the figures for net export of cereal grain, in millions of metric tons, 'then' (1948-1952) and 'now' (1974) are: Africa, −0.4 and −6.9; Asia, −5.8 and −47.1; North America, +22.5 and +76.4; Latin America, +1.2 and −0.4; Europe, −20.8 and −28.5 (United Kingdom, −6.9 and −7.3); USSR, +2.1 and +0.7; Oceania, +3.2 and +6.9.

These and other similar figures underlie the UN projections for annual growth in food supplies and in population until 1985 (see Murdoch and Oaten, *BioScience*, **25**, 561; 1975). For Africa, the projection is for 2.5% per annum growth in food supplies and 2.9% in population, for Asia (excluding China), 2.4% in food and 2.6% in people; for Latin America, 2.9% and 3.1%. Summing over all 'rich countries', the projections are for 2.8% per annum growth in food supplies and 0.9% in population; for 'poor countries', 2.6% and 2.7%. These trends are disturbing. They are made more so by the facts that the projections ignore rising energy costs, that a large fraction of the world population is malnourished at present and that the past UN projections have consistently erred towards optimism. □

Multiple controls in bacteria

from John Scaife

THE early choice of bacterial fermentation systems for gene control studies was a fortunate one. They prove to be excellent models of multiple gene control, readily analysed both *in vitro* and *in vivo*. The control of the lactose (*lac*) operon for example has two known components: a repressor protein and a protein termed CRP. The repressor, which directly governs the bacterium's response to β-galactosides, blocks the binding of RNA polymerase to its site in the promoter where transcription is initiated. CRP actively facilitates this binding step (see Gilbert in *RNA Polymerase*, edit. by Losick, R., and Chamberlin, M., Cold Spring Harbor Laboratory, 1976) using 3′, 5′-cyclic AMP as a cofactor. It thereby mediates in the *lac* operon the repressive effect of glucose (catabolite repression) to which many degradative operons are subject. Glucose modulates the amount of cyclic AMP in the cell.

Within the *lac* promoter the CRP-binding sequence (Beckwith, Grodzicker, and Arditti, *J. molec. Biol.*, **69**, 155; 1972) has now been identified among promoter fragments *in vitro* (Majors, *Nature*, **256**, 672; 1975). It lies upstream from the polymerase binding site. Interestingly the normal polymerase site in *lac* deviates significantly from other sequenced polymerase sites (Pribnow, *Proc. natn. Acad. Sci. U.S.A.*, **72,** 784; 1975). There is, however, a mutant, *UV5*, whose sequence is more in line with the others (see Gilbert, *op. cit.*). This strain does not require CRP for efficient *lac* function. The lesson is clear; CRP compensates for otherwise poor interactions between RNA polymerase and the normal *lac* promoter. How it compensates remains an unanswered question of great interest.

This well-founded and simple view of catabolite repression may nevertheless be incomplete. There is evidence that it might be mediated by more than one mechanism (Ullman, *Biochem. biophys. Res. Commun.*, **57,** 348; 1974). The behaviour of the galactose (*gal*) operon of *E. coli* has appeared particularly paradoxical. *In vitro* studies (Nissley, *et al.*, *J. biol. Chem.* **246,** 4671; 1971) clearly implicate CRP in *gal* function, yet mutants lacking CRP or cyclic AMP give good *gal* expression *in vivo* (Rothman-Denes, Hesse, and Epstein, *J. Bact.*, **114,** 1040; 1973).

New evidence for a second system mediating catabolite repression has just appeared. (Ullmann, Tillier, and Monod, *Proc. natn. Acad. Sci U.S.A.*, **73,** 3476; 1976) have partially purified from *E. coli* a new factor (CMF) which represses catabolite-sensitive operons in growing bacteria. The factor could resolve some of the present anomalies. It is unlikely to act by degrading CRP or cyclic AMP. It could interfere with inducer uptake or affect transcription directly.

There is, however, a different explanation for the *gal* paradox. It is suggested by features of the newly elucidated *gal* promoter sequence (Musso, Di Lauro, Rosenberg, and de Crombrugghe, *Proc. Natn. Acad. Sci. U.S.A.*, in the press). The *gal* operon has in its promoter binding sites for CRP and RNA polymerase like those of the *lac* operon. In *gal*, however, there are two start points for transcription. One of them uses CRP and cyclic AMP; the other does not, and in fact is inactive in their presence. Thus Rothman-Denes and coworkers were probably observing the CRP-independent mode of *gal* transcriptions.

Why should there be two transcription modes in this operon? The bacterium uses galactose in two quite different ways—as a carbon source and a cell-wall component—providing two metabolic contexts for *gal* enzyme synthesis. How they are distinguished can now be understood. Galactose as a carbon source induces the operon by inactivating the repressor. Musso *et al.* show that this requires transcription in the CRP mode, since the *gal* repressor hinders the binding of CRP, not polymerase. Without exogenous galactose, however, the operon must remain partially active to permit growth. This requirement can be satisfied by transcription, in the CRP-independent mode, despite the active repressor in the cell. The way is now open to tackle the challenging problem of how CRP-independent *gal* transcription is related to the growth process. Could it involve CMF? □

Fusion in Berchtesgaden

from J. G. Cordey

The sixth International Conference on Plasma Physics and Controlled Nuclear Fusion Research was held at Berchtesgaden in Bavaria on October 4-15, 1976. A more detailed account, which includes progress in fields of reactor design and inertial confinement (not discussed here) will be published soon in *Nuclear Fusion.*

DURING the past few years significant progress towards achieving thermonuclear fusion conditions has been made using the tokamak approach. This has been mainly due to the considerable effort that has gone into this field, which to some extent has been at the expense of the other containment geometries. The plasma physics behaviour in a tokamak is not yet fully understood, but experimental scalings at present suggest that fusion conditions can be obtained in a modest sized experiment. As far as the other containment approaches are concerned the route to a reactor is less clear.

Since the last meeting in this series, held in Tokyo in 1974, several new experiments have been commissioned. In particular the first results from two new large toroidal experiments T-10 and PLT were presented. Both these experiments are of the tokamak type, PLT (Princeton Large Tokamak) is at the Princeton Institute of Plasma Physics in the USA and T-10 at the Kurchatov Institute in the USSR. The machines have similar dimensions, the major radius of the torus in T-10 is 150 cm (PLT, 130 cm), the minor radius 39 cm (PLT, 40 cm). The toroidal field was operated at 3.5 T in both devices. The discharge current in these devices was 0.4 MA in T-10 and 0.59 MA in PLT. This current, which is in the toroidal direction, is essential for the formation of a plasma equilibrium and also heats the plasma by ohmic dissipation.

The parameters of the plasma contained in the two devices were broadly similar; the electron temperature in the centre of T-10 was 1.2 keV (1.9 keV in PLT), the ion temperature 0.6–0.8 keV (0.9 keV in PLT), the plasma density (n) 8×10^{13} cm^{-3} (3.5×10^{13} in PLT). The major difference between the two experiments was in plasma purity. In T-10 the plasma was rather pure having an effective charge between 1 and 2 whereas in PLT the effective charge was between 5 and 7. The energy containment time τ in T-10 was 0.06 s (0.04 s in PLT), giving an $n\tau$ (the Lawson product) of 5×10^{12} cm^{-3} s. Although the value of $n\tau$ and the ion temperature are both short of what is required in a reactor ($n\tau \sim 10^{14}$ cm^{-3} s and an ion temperature of 10 keV), they are a substantial improvement on the results obtained in smaller machines operated with the same magnetic field.

There was much discussion about the scaling of the energy confinement time τ between these larger experiments and the smaller experiments. The general consensus was that the energy containment time was scaling as the minor radius to the power α ($\tau \propto a^{\alpha}$) with α lying somewhere between 1.5 and 2. This result is quite encouraging and provided this type of scaling extends into the higher temperature region needed in a reactor, then it should be possible to achieve reactor conditions in a tokamak of modest size.

Moving on to the smaller tokamak experiments, the strong scaling of $n\tau$ with magnetic field strength has been demonstrated in the Alcator tokamak at MIT. Alcator is a small tokamak, major radius 54 cm, minor radius 9 cm, but has been operated with very high magnetic fields up to 8 T. At the highest magnetic fields, densities of 7×10^{14} cm^{-3} and containment times of 0.02 s have been achieved giving an $n\tau$ of 10^{13} cm^{-3} s. Thus it seems that high magnetic fields have a significant effect on the plasma containment and allow operation at higher density.

The neutral injection heating of tokamaks has continued to be successful. Ion temperatures up to 2 keV have been obtained in TFR and Ormak, and the increase in ion temperature is proportional to the injected power. The ion energy confinement time seems to be independent of temperature and does not follow the neoclassical scaling law at these higher temperatures.

The initial experiments on the DITE tokamak at Culham using the 'Bundle Divertor' are also quite encouraging. The purpose of a divertor is to remove impurities from the edge of the plasma. (Impurities poison the plasma and lower the temperature of the edge region.) The bundle divertor consists of two coils whose magnetic field distorts the main toroidal field such that the field lines at the edge of the plasma are diverted into an external pumping chamber before returning to the torus. The divertor on DITE is only operating at low toroidal field at the moment, but has successfully reduced the influx of gas and impurities to the plasma; as yet there has been no increase in electron temperature however.

A complete theoretical explanation of tokamak containment is not yet available, but present indications are that in the middle of the device the containment is determined by magnethydrodynamic instabilities and in the outer region is lost mainly by radiation. In the intermediate region drift wave turbulence is thought to be responsible for the energy loss. Measurements of the amplitude of the drift wave spectrum in the TFR tokamak at Fontenay however, indicated that it was not sufficient to account for the thermal conduction in this region.

Turning to other containment devices there was a revival of interest in stellerators at the conference. Results were presented from three new stellerator experiments—W7A at Garching, L2 in the Lebedev and Cleo at Culham. All these machines are smaller than the present generation of tokamaks, with minor radius of around 10 cm. Their energy containment time was typically between 1 and 8 ms with electron temperatures up to 800 eV. These results are clearly an advance over those obtained in the model C stellerator experiment at Princeton some years ago and are comparable with tokamaks of similar size and field strength. In fact these machines can be run in either a pure tokamak mode by not energising the stellerator helical windings or a stellerator–tokamak hybrid mode. Comparisons were made between the two types of operation and it was found that with the stellerator field on, the containment time was increased. Thus it seems that the extra complication of the stellerator helical winding can give worthwhile improvement in containment. I think, however, that the difficulty of designing suitable helical windings for large-scale devices with stronger magnetic fields may restrict progress in this field.

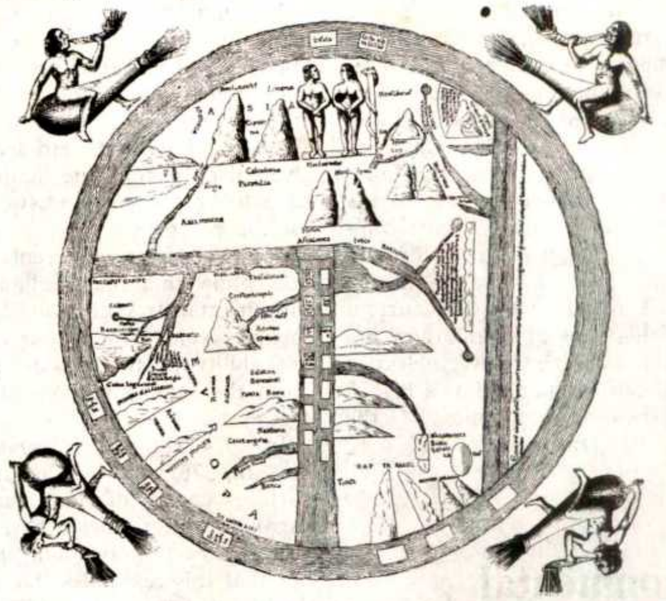
Both the tokamak and the stellarator are toroidal containment devices and in these the magnetic field lines stay within the plasma volume. There is another class of linear containment device however, and in these the force lines leave the plasma volume at the ends. The most important member of this class is the mirror machine, and in this device the plasma is trapped in the magnetic well formed between two coils, the particles 'mirroring' between the high-field regions close to the coils.

There has been a considerable increase in understanding the physics of mirror machine containment in the past 2 years, mainly due to experimental work on the 2XIIB machine at Livermore. Using very powerful neutral injectors delivering in total 5 MW of power at an energy of 20 keV, plasma densities of 10^{14} particles cm^{-3} at a mean ion energy of 13 keV have been built up from a target gas. To suppress the amplitude of ion cyclotron microinstabilities a warm plasma stream is injected along the field lines; unfortunately this stream also cools the electrons. In fact the electron temperature was less than 100 eV and this low electron temperature resulted in the rapid cooling of the injected ions; the energy containment time was typically of the order of 0.001 s giving an $n\tau$ of 4×10^{10} cm^{-3} s. The average value of β, that is the ratio of plasma pressure to the magnetic pressure, was very high —of the order of 0.7. This may be compared with tokamak β values which are typically of the order of 0.01, so the mirror machine is very economic in the use of magnetic field. It is this, along with its simplicity, that makes the mirror machine attractive from the reactor designer's point of view.

The basic problem with the mirror machine, however, is the rapid loss of plasma through the ends of the device. As well as losses caused by classical Coulomb collisions, there are additional losses due to microinstabilities which are driven by the anisotropy of the velocity distribution of the mirror-contained plasma. There are good theoretical arguments for expecting these microinstabilities to be less important in reactor scale devices with larger radius. But even if the only losses were classical, a self-sustaining mirror reactor would not be possible, and the device would have to be operated as an energy amplifier. Unfortunately the energy amplification factor Q is rather low, of order 2, and several schemes have been advanced for raising Q to around the 10 or 20 required for an economic mirror reactor. At this conference a novel triple mirror scheme was proposed by Dimov *et al.* (IAEA Conference Berchtesgaden paper C4). The idea is to place a small mirror machine at each end of a large mirror machine. The temperature and density in the outer mirror machines were maintained by high energy (~ 1 MeV) neutral injection at a higher level than the temperature and density of the main inner miror machine. The resulting ambipolar potential of the outer mirror machines reflected the ions in the inner mirror thereby improving their containment. By suitably adjusting the ratio of the lengths of the central mirror machine to outer mirror machines, it was thought that Q values in the region of 5–10 could be obtained.

Fortunately, no basically new types of instability have been found during recent theoretical work and most of the theoretical papers were concerned

A hundred years ago



Tenth century map of the world exhibited at the International Geographical Congress in Paris. From *Nature* **15**, January 11, 234; 1877.

with analysing the effects of existing modes in more realistic geometry. There was also an interesting and welcome trend in that theoreticians were attempting to compare their predictions with experimental results.

The Oak Ridge theoretical group gave an analysis of the flux-conserved tokamak equilibrium. These equilibria are attained by increasing the plasma pressure in a time which is short compared with the magnetic field diffusion time. In this manner β values of the order of 0.3 are achieved. The current profile of this type of equilibrium has not yet been examined; nevertheless, if a tokamak can be operated at a high β then the reactor economics of the device will be greatly improved. ☐

Environmental stress cracking

from P. D. Calvert

WHEN the bottom of a polyethylene bucket splits after six months use the cause is probably environmental stress cracking. The action of detergent solutions and residual stresses from the moulding process cause the slow growth of a crack which usually follows the boundaries of the polyethylene spherulites. Fifteen years ago this was a matter of great industrial concern as coverings on underground cables were prone to crack at bends. It was found, however, that the problem could be largely eliminated by suitable control of the grade of polymer and moulding conditions and the level of industrial interest decreased. But some progress has since been made on the more academic question of why this cracking occurs.

The phenomenon of environmental stress cracking has been extensively reviewed (see Howard, *Soc. Plastics Eng. J.*, 397; 1959; *Engineering Design for Plastics* (ed. Baer, E., Krieger, Basel; 1964)). Cracking is sensitive to the molecular weight distribution of the sample and in particular cracking resistance is improved by removal of the lowest molecular weight species (Herman and Biesenberger, *Polymer Engineering Sci.*, **6**, 341; 1966), which probably segregate to the spherulite boundaries and weaken them. Tests for stress cracking measure the time to fracture of a sample either bent and immersed in a liquid, or held under constant load in the liquid.

High density polyethylene is more resistant than low density to stress cracking under a constant load, but the situation is reversed when bent samples are tested. Fire polishing improves resistance to stress cracking, presumably by removing small surface flaws.

The liquids which cause stress cracking in polyethylene tend to be of low viscosity and to be surface active. Non-ionic detergents are quite effective, as are silicone oils, mineral oils and alcohols, but not ethylene glycol, glycerol or water. The liquid does not have to be a solvent or swelling agent for the polymer.

From a series of recent studies we now know that the mechanism of environmental stress cracking at low loads, involves void growth and depends on the ability of the liquid to penetrate from the sample surface to the crack tip.

In 1974 Hannon (*J. appl. Polymer Sci.*, **18**, 3761) showed that the fracture surfaces consist of small smooth areas surrounded by zones where the polymer has been drawn out. He pointed out that this resembles the situation in ductile fracture surfaces of metals where fracture starts by the growth of voids around particulate inclusions and ends when the voids grow large enough to join up. In polyethylene the voids must start at locally weak regions. Addition of detergent to the testing environment results in larger voids with less deformed material. Berg (*J. Res. NBS*, **76C**, 33; 1972) discussed theoretically the process of cavity growth in metals and suggested that this could be applied to stress cracking in polymers.

At high loads the crack velocity can become sufficiently large that the progress of the crack tip is limited by the ability of the liquid to flow in. On this basis Williams and Marshall (*Proc. R. Soc.*, **A342**, 55; 1975) made a fracture mechanics analysis of stress cracking and predicted a change in the stress dependence of crack speed, which they observed to occur at about 3×10^{-7} m s^{-1} in a detergent. Shanahan and Schultz (*J. Polymer Sci.*, (*Phys*), **14**, 1567; 1976) have now established this change by measuring fracture times of polyethylene samples in a series of silicone oils of different viscosity. At low loads the time to failure is independent of viscosity but for each viscosity a critical rate is reached above which the failure time changes only slowly with load. This critical time to failure is roughly proportional to the fluid viscosity. The most obvious interpretation of this is that the total time to fracture is the time it takes the crack to grow, but Shanahan and Schultz make the rather more elaborate assumption that when, by creep, a critical strain of 15% is reached, fracture occurs rapidly at a rate proportional to the creep rate. This, however, is a point which ought to be easily cleared up.

Although this gives us a good idea of how the fracture occurs it does not help to explain why polyethylene is the only polymer in which it happens and why some liquids are more effective than others. Spendal (*J. appl. Polymer Sci.*, **16**, 2375; 1972) has shown that the addition of 10% rubber can dramatically improve stress crack resistance, presumably by toughening the boundary regions. With rather more subtlety Schonhorn *et al.* (*J. Polymer Sci.* (*Phys*), **11**, 1013; 1973) have shown that the addition of silanes to polyethylene can improve stress crack resistance and base their arguments on the ways in which the interfacial energies of polymer–polymer and polymer–liquid are modified.

There are many other cases where polymers do form voids under stress both with and without environmental agents; the phenomenon is generally called crazing. Why this should be so common amongst polymers and why only polyethylene is vulnerable to the peculiar form of voiding discussed above is not known although Haward (*The Physics of Glassy Polymers* Applied Science, 1975) has discussed the question in some detail. But the solution to this problem is unlikely to help the humble plastic bucket. ☐

Serpentinite in the oceanic crust?

from Peter J. Smith

IT is now more than 14 years since Hess (in *Petrologic Studies*, Geological Society of America, 1962) suggested that serpentinised peridotite is the major constituent of seismic layer 3 of the oceanic crust. In the debate that has gone on throughout most of the intervening period, arguments have been adduced both for and against the proposal, although the balance of belief now seems to be against it. As the serpentinisation of peridotite can only occur below 500 °C, Hess envisaged the hydration of upper mantle peridotite taking place upon the latter's moving upwards through the 500 °C isotherm beneath an oceanic ridge; but as Fowler (*Geophys. J.*, **47**, 459; 1976) has recently pointed out, the depth and proximity of layer 3 to the ridge axis combined wth a reasonable thermal model of the crust militate against widespread serpentinisation. Moreover, as Cann (*Geophys. J.*, **15**, 321; 1968) has noted, the very small scatter of layer 3 seismic velocities would imply an unacceptably high uniformity of serpentinisation throughout the layer.

Cann's alternative suggestion is that the lower oceanic crust consists largely of gabbros and metagabbros. But even so, it does not follow that serpentinite plays no part at all in crustal formation. On the contrary, it is evident that serpentinite bodies are closely associated with the major tectonically active fracture zones offsetting ridge axes. For example, the steep south wall of the Vema fracture zone which offsets the mid-Atlantic ridge by 320 km at 11°N is the edge of a serpentinite intrusion running parallel to the fracture and thus orthogonal to the ridge. And similar bodies, probably emplaced diapirically from the upper mantle, are also found in conjunction with offsets of the Indian ridge.

But serpentinisation in association with major fractures is a special case in that it is localised. Does serpentinised peridotite play any significant part in the formation of 'normal' oceanic crust along ridges? Bonatti (*Earth planet. Sci. Lett.*, **32**, 107; 1976) now suggests that it does—not in the form of a continuous layer *à la* Hess but as vertical solid intrusions, or 'protrusions', and associated subhorizontal sills. The proposal here is that serpentinite bodies resulting from the hydration of upper mantle peridotite "move gradually upwards into the crust by solid diapirism during the initial stages of crustal spreading, and become emplaced along narrow bands parallel to ridge axes". By 'narrow' Bonatti means of the order of a few hundred metres; and the bands lie between the supposedly faulted blocks of basalt (layer 2) and gabbros/metagabbros (layer 3) which make up the bulk of the crust.

There is certainly evidence for the presence of serpentinite in the oceanic crust away from major fracture zones, at least in the Atlantic. For example, Aumento and his colleagues (see Aumento and Loubat, *Can. J. Earth Sci.*, **8**, 631; 1971) have reported that serpentinite outcrops are common near the mid-Atlantic ridge at 45°N. Numerous serpentinite samples have also been dredged from the north wall of the Vema fracture at 11°N. The latter, being found close to a major fracture, could be the result of fracture zone volcanism and thus irrelevant to the point at issue, although Bonatti offers convincing evidence for regarding the north wall of the Vema fracture (in contrast to the south wall) as a section of 'normal' crust. In any event, serpentinite samples from both sites share characteristics; they are restricted to no single crustal layer, they become shallower with distance from the ridge axis and they do not occur within a few tens of kilometres of the axis.

All of which would seem to support the idea of serpentinite diapirism. As Bonatti envisages it, the serpentinisation is due to sea water which both acts as hydrating agent and keeps the lower crust below the 500 °C isotherm. The process begins at the fractured margins of the ridge axial zone and extends laterally away from the ridge for perhaps 100–200 km. Vertically, it occurs to depths of several kilometres, probably even down to the crust–mantle boundary. The upward mobility of serpentinised mantle is made possible by the existence of deep dip-slip faults parallel to the ridge axis (the result of block faulting) and the low strength and density of serpentinite compared with other crustal rocks. Finally, assuming a ridge spreading velocity of 1.2 cm yr^{-1}, Bonatti estimates the rate of ascent of the serpentinite diapirs to be about 1 mm yr^{-1}.

The serpentinite diapirs proposed are too narrow to be detectable seismically, but there may be other direct and indirect ways of deducing their presence. For example, serpentinites often have high magnetisations, probably due to the formation of secondary magnetite, and thus give rise to strong magnetic anomalies. If the Bonatti thesis is correct his oceanic diapirs should give rise to narrow anomalies superimposed upon the wider Vine-Matthews anomalies; and since the diapirs supposedly enter crust already thousands to millions of years old, their magnetic polarity should not necessarily agree with that of the surrounding crust. In fact, short-wavelengths linear anomalies of the suggested type have already been reported by several workers although unfortunately there are other equally possible explanations for them.

Perhaps the best test, however, is direct sampling. Serpentinite bodies in the oceanic crust have already been encountered on several occasions during the work of the Deep-Sea Drilling Project. Sub-parallel strips of intrusive serpentinised ultramafics of the order of 100 m wide have also been found in gabbros and basalts in a part of Macquarie Island believed to be a section of uplifted Cenozoic oceanic crust. There must surely be other examples.

Energetic nuclear collisions

from P. E. Hodgson

When two nuclei collide at high energies there is a very strong interaction and many complicated reactions take place. The nuclei can excite each other and exchange a few nucleons before separating again, or they can fuse together for a short while to form a compound system that decays by emission of nucleons and alpha particles. At higher energies a nuclear shock wave can develop (*Nature*, **258**, 570; 1975) and this surges rapidly through the nucleus and causes the emission of a spray of nuclear fragments.

Some progress has already been made in understanding these reactions using the classical concepts of viscosity and the mechanics of shock waves, but further progress requires a more detailed quantum-mechanical treatment. The motion of the nuclei must be described in terms of the coordinates of their constituent nucleons by wavefunctions satisfying the Schrodinger equation.

Many theories of nuclear structure have now been developed that treat the motion of the constituent nucleons in this way, in particular the Hartree–Fock self-consistent field theory. This gives a series of coupled equations for the nucleon wavefunctions that can be solved in principle to give the single-particle wavefunctions and all the properties that can be derived therefrom. This is a very complicated calculation that can only be carried out in practice by making several simplifying approximations.

But the problem of understanding a nuclear collision in a similar way is immensely more complicated, essentially because it is dynamic. Although a time-dependent Hartree–Fock theory has been developed, it is difficult to apply it to nuclear collisions.

Faced with such a complicated problem, the best way to proceed is to make a simple model that, it is hoped, contains many of its main features, but is practicable to solve. Comparison between the solutions of the simple problem and the experimental data will then indicate the usefulness of the model, and possibly suggest ways of improving it. At the same time increasing familiarity with the mathematical structure of the simple solution may make it easier to see how the model can be made more realistic without at the same time making it impossible to solve.

This approach has recently been used by Bonche, Koonin and Negele (*Phys. Rev.*, **13**, 1226; 1976) in a first attempt to make a quantum-mechanical theory of the energetic interactions of heavy ions. To make the calculations tractable, they made very drastic assumptions, and yet rather surprisingly the results showed many of the features of the experimental data and thus encourage the development of the theory in more detail.

The first great simplification was to assume that the whole interaction essentially takes place in one dimension. They thus considered the collision of two slabs of nuclear matter having a specified thickness in their direction of motion but extending indefinitely in the directions at right angles. They also assumed that these slabs were completely symmetric in spin and isospin.

The Hartree–Fock theory requires the interaction between two nucleons, and this is known to be very complicated. They therefore constructed a simplified form of the interaction that contains most of the physics and adjusted its parameters to give the best overall agreement with nuclear properties.

In its full generality, the time-dependent Hartree–Fock theory gives a set of coupled non-linear integro-differential equations in three dimensions for the wavefunctions of the nucleons. After making the above approximations they reduce to a set of coupled linear equations that are readily solved by existing electronic computer techniques.

These equations were solved for a series of different initial conditions. They first of all studied the oscillations of a single slab, and found that these could take place at certain definite frequencies. These oscillations correspond to the collective 'breathing mode' oscillations of nuclei, that is oscillations without change of shape. The frequencies of such oscillations depend on the nuclear compressibility (see *Nature*, **254**, 480; 1975).

Next they found out how a slab interacts with a potential, and in particular how it is affected by passing through a potential barrier. At a speed $E/A = 10$ MeV and barrier of Gaussian form and height equal to the incident kinetic energy there is hardly any reflection, and the transmitted slab has a different density profile showing that it is in an excited state. The excitation consists of density ripples moving through the slab, and calculations show that 36% of the incident kinetic energy is converted into such excitations. This behaviour is markedly different from that of a single particle encountering a barrier at comparable energies: in that case only half the incident wave is transmitted and half is reflected. This comparison shows the importance of collective effects in the slab interaction; the attractive single-particle potential due to the other nucleons moving through the barrier reduces the effective potential and hence favours transmission over reflection.

As the barrier height is increased, so is the proportion of the slab that is reflected, until when the barrier is twice the incident kinetic energy it reaches 71%. In addition, the transmitted and reflected portions each split up into two pieces, one slowly moving and the other rapidly moving.

These studies are a useful preliminary to the main object of the calculations, namely to calculate the interaction of two nuclei, here represented by two slabs. At low energies ($E/A \approx 0.5$ MeV) the slabs fuse together to form a compound slab of rather uniform density that continues to oscillate without breaking up (see *Nature*, **261**, 193; 1976).

At a somewhat higher energy ($E/A \approx 3.5$ MeV) the compound slab is formed and then almost immediately separates again. Each fragment is oscillating strongly after the interaction, showing that it is highly excited. This process corresponds to the deep inelastic scattering of heavy ions, when two nuclei are strongly excited in a collision but essentially retain their identity, apart perhaps from the exchange of a few nucleons (see *Nature*, **256**, 261; 1975).

At much higher energies ($E/A = 25$ MeV) some quite new phenomena appear. As shown in Fig. 1, a region of very high density is formed, and from this region shock waves propagate outwards in both directions: these are indicated by the waves moving outwards at velocities higher than the incident slabs. When these waves of higher density reach the surface of the slab they separate from it, forming separate fragments that propagate rapidly outwards. Secondary waves follow them, and the region of almost uniform density that is formed for a short time in the central region breaks up from both ends into separate waves that also travel outwards and separate when they reach the surface, until eventually the whole compound system has broken up into fragments. Thus the model shows very well the phenomena of shock waves and fragmentation that take place in very energetic nuclear collisions.

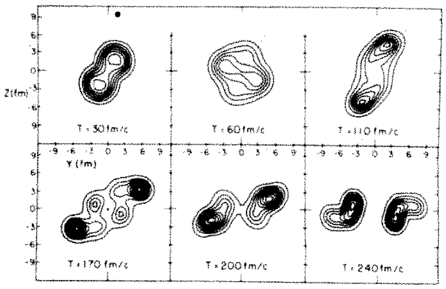


Fig. 2 Contours of the nuclear density for the collision of ^{16}O with ^{16}O at $E/A = 24$ MeV and an impact parameter of 4 fm. The contours are in the scattering plane and the density is integrated over the co-ordinate perpendicular to it.

An important advance has recently been made by Gusson, Smith and Maruhn (*Phys. Rev. Lett.*, **36**, 1166; 1976) in the form of a time-dependent Hartree–Fock (TDHF) calculation of the reaction $^{16}O + ^{16}O$ in three dimensions. The TDHF model they used assumes that the time-dependent wave function is given by a single Slater determinant whose occupied single-particle orbits obey the TDHF Schrodinger equation. For the interaction between the particles they used a simplified form of the Skyrme interaction, and assumed spin and isospin saturation, so that the spatial wave function applies to alpha particles.

Some of their results are shown in Figure 2, which gives the contours of the densities of the two interacting ions at various times in the plane of the collision, integrated over the direction perpendicular to that plane. The time scale is in units of fm/c, that is, about 3×10^{-24} s so the whole interaction takes about 10^{-21} s. These contour plots show that when a collision takes place at an impact parameter equal to about half the radius of the ions the outer regions interact strongly and stick the ions together in a dumb-bell shape which rotates about its centre of mass. As the system rotates, complicated internal motions take place, some parts rotating more rapidly than others. At one stage ($T = 170$ fm/c) small regions of higher density corresponding to alpha particles form in the region between the main mass concentrations. These are later attracted back and a neck forms between the two ions. The neck narrows and finally breaks, and the particles separate. They are now irregular in shape, indicating that they have been highly excited by the collision.

At no time in this interaction are high densities observed, indicating that the energy is too low for compression and shock wave phenomena to develop.

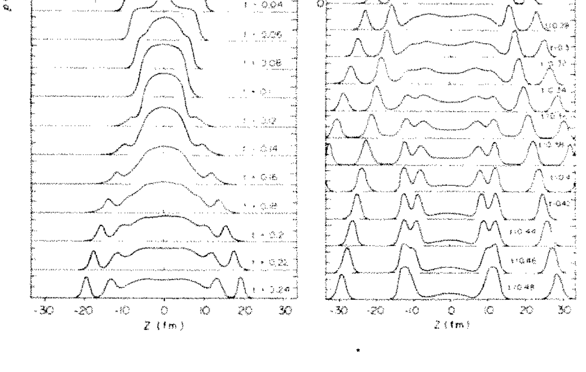


Fig. 1 Density profiles at various times t for the collisions of two slabs at a velocity corresponding to $E/A = 25$ MeV, showing the formation of a shock wave that propagates rapidly outwards, followed by a general fragmentation. (Times in units of 10^{-21} s.)

articles

New data on the lower Palaeozoic sequence of northern Victoria Land, Antarctica, and its significance for Australian–Antarctic relations in the Palaeozoic

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*New stratigraphical and palaeontological data from the Early Palaeozoic Bowers Group of northern Victoria Land indicates that its depositional basin was probably closely linked with the Dundas Trough of western Tasmania. On the **basis of these data, a revised reassembly of the Australo-** Antarctic portion of Gondwanaland is proposed.*

NORTHERN Victoria Land is a critical element in the reconstruction of the south-western Pacific sector of Gondwanaland. Most recent reconstructions of this sector have been based either largely on morphological best fits of continental margins[1–6] or on 'plate tectonic' reconstructions, that is, by 'reversing' seafloor spreading using a mapped pattern of magnetic lineations and fracture zones[7–11]. Only superficial attention has generally been given to the geology of the areas thus juxtaposed, primarily because of the lack of precise data from Antarctica.

Detailed evidence of distinctive structural–sedimentary belts that cross the Antarctic continental margin and thus serve as potential 'geological ties' has been singularly lacking, although broad correlation of Early Palaeozoic sequences has been attempted[2–4,7,12].

This note presents new stratigraphical, sedimentological and palaeontological data from northern Victoria Land which are inferred to provide such a tie in the Cambrian–Ordovician, linking the Bowers Group depocentre in Antarctica with the Dundas Trough of Tasmania. Data from Lower Palaeozoic sequences elsewhere in Antarctica and from the Adelaide Geosyncline support a continental reassembly constructed on this basis, and further suggest that Cambrian sedimentation in this segment of Gondwanaland may have occurred in separate eastern and western belts of contrasting environment. It seems that the widely accepted 'computer fit' of Australia and Antarctica[1] is unlikely to hold, at least during the early Palaeozoic.

Bowers Group sequence, northern Victoria Land

All known Lower Palaeozoic sedimentary rocks of northern Victoria Land are included within the Bowers Group[13], which lies within a 20–25-km wide infaulted belt extending south-east from near the mouth of Rennick Glacier for at least 270 km. To the north-east the Bowers Group rocks are inferred to be faulted against sediments of the Robertson Bay Group of probable Late Precambrian age[14]; and to the south-west they are faulted against metamorphic rocks of the Precambrian Wilson Group.

The oldest of the three units comprising the Bowers Group is the Sledgers Formation, a dominantly marine sequence whose base is not exposed. The formation, of minimum thickness 3,000 m, consists of mainly angular unsorted basaltic breccia containing common fragments of pillow lava, and a few thin flow units in the north; to the south it interfingers with dark mudstone containing thin interbedded rippled fine sandstone and lenticular turbidite units of graded coarse volcanogenic sandstone and conglomerate[15]. The sequence has yielded no age-diagnostic shelly fossils.

Overlying the Sledgers Formation without apparent discordance is the Mariner Formation, a fossiliferous marine sequence comprising sandstone at the base and passing upwards into shale with thin limestone and sandstone beds. The youngest beds of the Mariner Formation are red sandstone and mudstone with dessication cracks, rare inarticulate brachiopods and abundant trace fossils, including *Rusophycus* and *Cruziana*. This part of the sequence has been inferred[16] to represent a marine regression, the youngest beds representing an intertidal environment. Shelly fossils (trilobites and brachiopds) are relatively common throughout the Mariner Formation, the basal beds being of late Middle Cambrian age (*Ptychagnostus nathorsti* Zone or *Lejopyge laevigata* Zone) and the youngest fossils being of middle or late Late Cambrian age. At the southernmost limits of known exposure the formation is at least 1,600 m thick, but in the north of the mapped area, where the upper beds have been removed by Late Cambrian erosion, it is only 100 m thick.

A regional unconformity truncates the Mariner Formation, the overlying fluvial deposits of the youngest unit of the Bowers Group resting with marked erosion but no apparent angular discordance on middle to late Late Cambrian intertidal deposits in the upper Mariner Glacier region, and in places with marked discordance on early Late Cambrian marine mudstone 170 km to the north[17]. This youngest unit is exposed in two separate strips forming the eastern and western margins of the Bowers Group outcrop. In the eastern strip at least 3,000 m of red–brown or yellow quartzose sandstone, with minor quartzose conglomerate and mudstone (Camp Ridge Quartzite) is exposed in the core of a major syncline. The sequence is dominantly fluvial and lacks body fossils, although locally near the base trace fossils including *Rusophycus*, *Daedalus*, *Monocraterion*, and *Skolithos* occur[14]. In the western strip of outcrop, mainly fluvial deposits of minimum thickness 800 m dominated by conglomerate (Carryer Conglomerate) occupy a similar stratigraphic position unconformably above fossiliferous marine beds[15].

Late Cambrian and Early Ordovician granitic rocks crop out extensively to the west of the Bowers Group, but none are known to intrude it. Post-tectonic granitic plutons of Devonian to Early Carboniferous age (Admiralty Intrusives) intrude both the Robertson Bay and Bowers Groups. This igneous province is restricted to the area east of the Rennick Glacier, and is thought by some writers to be associated with tectonic movements (Borchgrevink Orogeny) which resulted in folding of both sedimentary groups[13,18].

Dundas Trough Sequence, Tasmania

The main locus of Cambrian sedimentation in Tasmania (the Dundas and Fossey Mountain Troughs) lay between the Precambrian Rocky Cape and Tyennan crustal blocks and the Forth Nucleus, forming an arcuate belt around the northern and western margins of the Tyennan Geanticline[19,20]. The axial portion of the Dundas Trough contains two main marine sedimentary sequences separated by an inferred unconformity[21]. The older sequence, which contains no shelly fossils, consists of greywacke, siltstone, shale and quartzite with some interbedded mafic volcanics (Crimson Creek Argillite, Success Creek Group, and correlatives[22–24]). Ultramafic complexes are found associated with these older rocks at several localities within the Dundas Trough, but are not known to intrude the fossiliferous Cambrian sequences.

The younger sequence comprises turbidite greywacke, siltstone, shale and conglomerate (Dundas Group and correlatives). It contains fossils (mainly trilobites) at several horizons; the oldest beds are Middle Cambrian (*Ptychagnostus gibbus* Zone) whereas the youngest beds range in age from early Late Cambrian (*Mindyallan* Stage) in the north[25], to late Late Cambrian (*Ptychaspis-Prosaukia* Zone) in the south.

Sediments of Middle to Late Cambrian age were also deposited in the Smithton Basin to the north-west of the Rocky Cape Geanticline[20], in the Dial Range Trough (a northern extension of the main Dundas Trough), and in the Adamsfield Trough to the east of the Tyennan Geanticline[26,27].

Along the eastern margin of the Dundas Trough, adjacent to the Tyennan Precambrian block is an arcuate belt of acid to intermediate volcanic rocks and associated sediments—the Mount Read Volcanic Arc. Rocks of the lower part of the sequence (Queenstown Pyroclastics, Central Lava Belt and Intrusives) grade westwards into greywackes of the Dundas Trough. The upper part of the sequence (Tyndall Group) unconformably overlies the lower part and is composed of tuff, agglomerate, sandstone and conglomerate; fossils of late Middle or early Late Cambrian age indicate that the Tyndall Group is, at least in part, of equivalent age to the Dundas Group to the west.

The Cambrian Dundas Group and Tyndall Group sediments are both unconformably overlain by the Owen Conglomerate or its equivalent, representing the basal unit of the Junee Group[21]. This contact marks a conspicuous change in lithology and sedimentation. The Cambrian units are predominantly of the siltstone–greywacke–greywacke conglomerate association, whereas the Owen Conglomerate and its equivalents are quartz- and chert-rich sandstone and conglomerate. Fossils of early Ordovician age are known from the middle part of the Junee Group and the unfossiliferous basal beds are likely to be of late Cambrian or earliest Ordovician age. Along the axis of the Dundas Trough contact between conglomerate and underlying beds is generally paraconformable[25]; however, successively younger beds underlie the contact from north to south, so that at Birch Inlet—the most southerly sequence—only a short break in sedimentation is inferred, whereas in the Dial Range Trough in the north, most of the late Cambrian is missing[25]. Near the northern margin of the Tyennan Geanticline three small granitic plutons of probable Late Cambrian age[28] intrude Cambrian and older rocks.

Shallow water platform sedimentation continued over much of eastern Tasmania from Ordovician to Early Devonian times without notable interruption until a widespread period of deformation between the Early and Late Middle Devonian, known as the Tabberabberan Orogeny, affected all the Early Palaeozoic rocks of the area. The Orogeny was accompanied and followed by granitic intrusions.

Cambrian sequences elsewhere

In Victoria, widely scattered outcrops contain sparsely fossiliferous Middle and Upper Cambrian shale and sandstone overlying an unfossiliferous sequence of spilites and cherts[29]. Unlike the intensively studied Ordovician, however, the Cambrian sequences are poorly exposed and the stratigraphic relationships not sufficiently well known to be discussed in detail here.

In South Australia Early Cambrian rocks rest disconformably on Late Precambrian sediments in the main area of sedimentation, the Adelaide Geosyncline[30]. The Lower Cambrian is characterised by a thick succession of shallow water shales and archaeocyathine-bearing carbonates. Extrusive igneous rocks are limited to a few small areas of basic to intermediate volcanics in the southern part of the Adelaide Geosyncline[31], and some thin tuffaceous horizons within the Cambrian sediments of the Flinders Ranges[32]. In the late Early Cambrian, uplift led to erosion of older Cambrian sediments in the Yorke Peninsula area and the formation of conglomerates on both Yorke Peninsula and Kangaroo Island[32]. Widespread carbonate deposition at the beginning of the Middle Cambrian was followed by red-bed deposition which may have extended up into the Late Cambrian in much of the Adelaide Geosyncline, including Yorke Peninsula[32], as fossiliferous late Middle and Upper Cambrian sediments are known from the Frome Embayment and the Warburton Basin further to the north.

In the Kanmantoo Trough, lying to the east of the Adelaide Geosyncline, a thick succession of metasediments (Kanmantoo Group) conformably overlies fossiliferous Early Cambrian sediments (Normanville Group). An inarticulate brachiopod, *Lingulella*, is found near the base of the Kanmantoo Group; however, with this exception, worm burrows are the only fossils known. The clear-cut contact between the Normanville and Kanmantoo Groups indicates the start of a new depositional cycle which was triggered by the Kangarooian Movements. These movements which affected the Fleurieu Peninsula, Kangaroo Island and Yorke Peninsula areas, lasted at least into the Middle Cambrian[33]. Late Cambrian and Early Ordovician granites intrude the Kanmantoo Group in several places, thus placing a maximum age range of Early to Late Cambrian on its time of deposition. Other granites of similar age are found in southern South Australia. A folding movement (Delamerian Orogeny) took place in the Late Cambrian–Early Ordovician[34].

In Antarctica, the nearest known fossiliferous Cambrian sediments to those in northern Victoria Land lie 1,000 km to the south in the region between the Nimrod and Byrd Glaciers. Here the Byrd Group[35] comprises over 8,000 m of limestones with minor shale, sandstone, quartzite, and conglomerate unconformably overlying Late Precambrian metasediments. Archaeocyathinae occur sporadically throughout most of the sequence, but have been positively dated only from immediately north of the Nimrod Glacier. Here a small collection from beds within 1,000 m to 1,300 m of the base of the Group belong to the Lower Lena Stage (late Early Cambrian)[36]. The stratigraphically highest Archaeocyathinae occur at least 6,500 m above the base of the Byrd Group, and are likely to be early Middle Cambrian.

South of the Nimrod Glacier widely separated outcrops of mixed clastic and carbonate sediments containing fossils of Early and Middle Cambrian age occur in the Shackleton Glacier[37] and Leverett Glacier[38] areas respectively. North of the Byrd Glacier, in south Victoria Land, scattered areas of unfossiliferous but probably in part Cambrian mixed carbonate–clastic sequences are also known[39]. No further outcrops of calcareous rocks correlatable with the Early and Middle Cambrian Byrd Group occur between south Victoria Land and the northern coast, but a hint that the archaeocyathine limestone belt, now eroded or covered by ice, may once have extended as far north as the coast west of northern Victoria Land, is given by the record of marble erratics, one containing a possible Archaeocyathid, at Cape Denison[40] (Fig. 1).

Early Ordovician granitic rocks, which intrude the Byrd Group

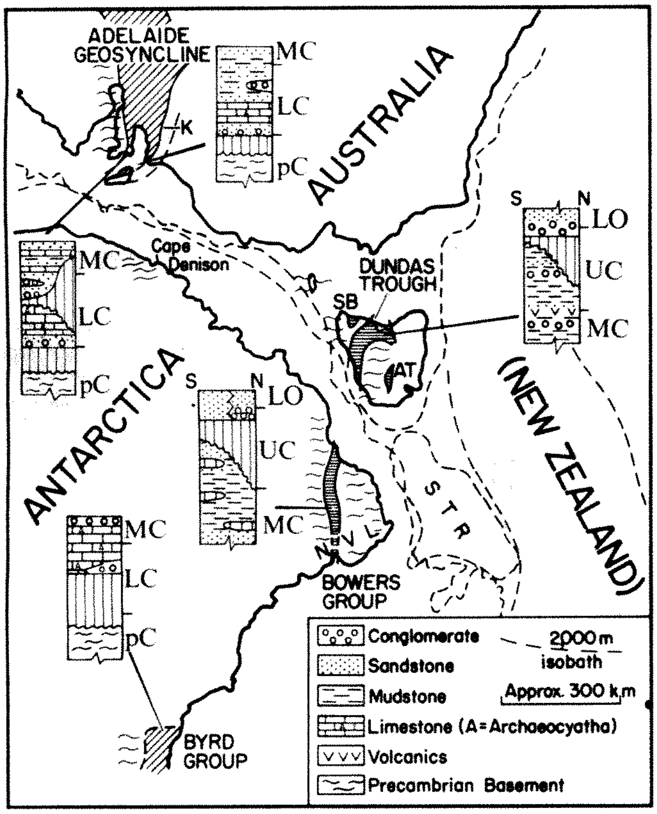

Fig. 1 Reconstruction of the south-eastern Australian and East Antarctic elements of Gondwanaland, showing the main Cambrian depositional sites (horizontally and obliquely hatched areas) and stratigraphic columns for critical successions. NVL, Northern Victoria Land; STR, South Tasman Rise; AT, Adamsfield Trough; K, Kanmantoo Trough; SB, Smithton Basin. Vertically hatched areas on the stratigraphic columns represent time gaps. Note: stratigraphic column for Dundas Trough is for the axial part, south to north, and thus does not include Mount Read Volcanics. The column for the Bowers Group similarly spans the belt from south to north. pC, Precambrian; LC, MC, UC, respectively Lower, Middle and Upper Cambrian; LO, Lower Ordovician.

south of the Nimrod Glacier[41], are widespread throughout the central Transantarctic Mountains and south Victoria Land. This intrusion of granites accompanied and followed a major tectonic movement, the Ross Orogeny, which was responsible for folding the Byrd Group and its probable correlatives.

Australia–Antarctica reassembly

Figure 1 shows comparative columns of the main elements of the stratigraphy and tectonics of the Cambrian sequences in Antarctica, South Australia, and Tasmania. A similarity is at once apparent between the northern Victoria Land (Bowers Group) and Tasmanian columns, constructed along the axial portions of both belts. The youngest fossiliferous marine deposits are in both cases overlain unconformably by dominantly continental coarse quartzose sandstones and conglomerates of probable latest Cambrian or earliest Ordovician age. They are both underlain by unfossiliferous clastic sequences with mafic volcanics; however, the volcanics form a major part of the Antarctic sequence but only a minor part of the Tasmanian succession. The Cambrian of both areas is intruded by Devonian–Carboniferous granites, in contrast to the other areas studied. Both successions were apparently deposited in rapidly subsiding trough-like structural depressions bounded by Precambrian blocks. Both regions were affected to a similar degree by tectonic activity particularly during latest Cambrian and Early Ordovician times, resulting in similar sequences of quartzose gravels and sands. It is likely, therefore, that western Tasmania lay very close to the Bowers Group depocentre in Cambrian and Early Ordovician times.

By contrast, fossiliferous Late Cambrian is absent from the southern South Australian sequences, while the Early and early Middle Cambrian, which appears to be almost fully represented, contains prominent archaeocyathinae limestone horizons. The contrast between the stratigraphy and geological history of the Cambrian of northern Victoria Land and that of South Australia shows that the sequences could never have been directly continuous, and the areas were unlikely to have been adjacent. However, a similarity is apparent between the archaeocyathine limestone-bearing Byrd Group of Antarctica and the South Australian sequences.

A reasonably firm geological basis for continental reconstruction between Australia and Antarctica has thus been established. The "geologic fit" proposed here (Fig. 1) differs markedly from the widely accepted "computer fit" based on morphology[1], but is relatively close to Griffith's "plate tectonic" reconstruction[7]. However, in this fit, Australia has been rotated anticlockwise slightly with respect to Antarctica so that the Cambrian of western Tasmania is nearly continuous with a possible northern extension of the Bowers Group. This reassembly has the added advantage of accommodating the South Tasman Rise with almost no overlap adjacent to the eastern coast of northern Victoria Land. The similarity of the quartzose semi-schist of the Rise with that of the Robertson Bay Group, and the coincidence of K/Ar age dates in both areas, has already been noted and correlation suggested by other workers[42]. It is interesting to note that the proposed fit, based on correlation of the Cambrian of western Tasmania and of northern Victoria Land, leaves the Archaeocyathine limestones of the Adelaide Geosyncline and of the Byrd Group in a position apparently far to the west of the projection of the Bowers Group–Dundas Trough alignment. This outcrop distribution could be accounted for by post-Cambrian strike-slip movement between Tasmania and mainland Australia causing segmentation of a once continuous but somewhat sinuous belt (as some writers[43–46] have suggested), or pair of belts. An alternative possibility (rendered particularly attractive if Mawson's reported discovery[40] of a possible Archaeocyathid in marble erratics at Cape Denison represents the northern extension of the Byrd Group) is that Cambrian sedimentation occurred in two distinct belts, the Early and Middle Cambrian Archaeocyathid-bearing one lying parallel to but well to the west of the Bowers Group–Dundas Trough depocentre. If the latter interpretation is valid, the necessity for major strike-slip movements between Tasmania and mainland Australia is eliminated.

The general similarity between the Cambrian fit proposed here and Palaeocene reassemblies of Antarctica and Australia based on "reversal" of seafloor spreading[7, 8] is highly significant, implying that the relationship of the western Ross Sea sector of Antarctica to Tasmania and possibly to the whole of south-eastern Australia was substantially the same in the Cambrian and in the Palaeocene.

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Preliminary findings of the Viking gas exchange experiment and a model for Martian surface chemistry

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Oxygen and CO_2 were evolved from humidified Martian soil in the gas exchange experiment on Viking Lander 1. Small changes in N_2 gas were recorded. A model of the morphology and a hypothesis of the mechanistics of the Martian surface are proposed.

THE early results from the gas exchange experiment (GEX), on Viking Lander 1 have already been reported but were limited to preliminary findings only[1]. This paper examines the findings and interprets them in terms of a model of the soil surface morphology and chemistry.

All major GEX activities that occurred after landing up to the end of the first incubation cycle are given in Table 1. In Table 2 are listed the sample processing and transport environments to which the soil was subjected up to the point when the first analysis was made; they may have relevance to the extent and nature of the gaseous responses.

Data on instrument performance will be reported at a later date. Descriptions of the concepts governing the design of the experiment, modes of operation and results obtained are described elsewhere[2–7].

Humid mode results

The first incubation cycle began with the addition of 1 cm^3 Martian soil to the 8.7 cm^3 test cell (0.465 $cm^3 \pm 0.035$ cm^3 solid volume, estimation based upon solid density range of 2.6–3.0 $g\,cm^{-3}$ for the soil in the test cell and a bulk density of 1.3 $g\,cm^{-3}$) (Viking Physical Properties Team). In the process of loading the soil and sealing the test cell, Martian atmosphere was trapped within the chamber at the prevailing pressure of 7.6 mbar (ref 8). A 0.132 cm^3 volume of starting gas at 8.394 atm consisting of 5.51% Kr, 2.84% CO_2, and 91.65% He

Table 1 Major events in the first cycle of the gas exchange experiment

Event	Date (Sol)*	Elapsed time from landing (s)
Landing	0	0
Initiate chromatographic column rejuvenation	2	206,156
Soil sample added to test cell	8	682,426
Seal test cell	8	683,439
Add incubation gas, Kr/CO_2/He	9	759,219
Inject 0.57 ml nutrient (humid mode)	9	759,879
First headspace gas analysis (humid)	9	769,876
Second analysis	10	860,176
Third analysis	11	948,916
Fourth analysis	13	1,126,756
Fifth analysis	15	1,302,556
Inject 2.3 ml nutrient (wet mode)	16	1,383,339
First headspace gas analysis (wet)	16	1,392,916
Second analysis	17	1,481,656
Third analysis	18	1,570,456
Fourth analysis	20	1,744,456
Fifth analysis	25	2,189,320
Sixth analysis	28	2,446,105

The column rejuvenation cycle is the process by which the gas chromatograph columns, which have been opened to the vacuum of space, are cured of the need to be primed for oxygen in order to make quantitative measurements of oxygen in any given sample. The test cell had two positions on the carousel: the first to receive sample and the second to be sealed against the head-end. The latter contained all the attachments for adding gases and nutrient and for removing sample from the headspace. The soil was contained in a porous cup suspended over a sump that allows drainage, when required, and variable additions to either humidify or wet the soil. Test cell volume empty was 8.7 cm^3 and the sample loop volume was 0.1114 cm^3. The amount of gas lost by sampling varied as a function of the total pressure in the test cell, the relative sample loop and headspace volumes and the gas distributions which were a function of temperature and gaseous species.

*Sol (the Martian day) is 24 h, 39 min, 35 s long.

Table 2 Soil sample history

Location	Temperature (K)	Time duration (h)
Soil surface (0–5 cm deep)	190–208	N.A.
Collection head (collect then dump)	204–206	0.09
Arrival at Biology PDA (soil processor) through shuttle (dump from PDA to SDA)	239–280	0.26
Arrival at Biology SDA (soil distribution) through soil dump to test cell	280–282	0.05
Arrival of soil in test cell through seal of test cell	282	0.28
Seal of test cell through humidification (addition of 0.57 ml nutrient)	282	21.2
Humidification through first analysis	282	2.78

Soil temperature estimated, all other temperatures directly measured.

and 0.567 cm^3 of M4 nutrient[3] were added to the lower cavity of the test cell, an amount insufficient to wet the soil but providing 100% relative humidity. The final pressure in the test cell after the above additions is estimated to be 150 mbar. Results of the first and subsequent gas chromatographic analyses of the headspace gases during the humid mode are shown in Table 3. After analyses of the headspace, the total gases in the test cell were estimated from Ostwald coefficients for aqueous solutions[9–14] to determine the contribution of the gases dissolved in the liquid phase. The results were corrected for the initial contributions of the original trapped Martian atmosphere[15], the starting Kr/CO$_2$/He gases and Ne introduced by the nutrient injection and losses from sampling the headspace gas. Solution coefficients were used for CO$_2$ to correct for the estimated basicity of the soil (Table 3). This technique was used only for CO$_2$ to approximate the magnitude of the CO$_2$ change.

The results show the CO$_2$, O$_2$, N$_2$ and Ar and/or CO (the Ar and CO are not resolved by this gas chromatograph) were evolved by the soil sample. The maximum amount of N$_2$, 83 nmol, appeared on Sol 11 (Sol is a Martian day, equal to 24 h, 39 min and 35 s) and decreased to 77 nmol by Sol 15. Oxygen, on the other hand, after reaching its maximum on Sol 11, appears to have reached a plateau. From the assumed total oxidation of ascorbate in the M4 nutrient[3], the net amount of O$_2$ produced equals 775 nmol (690 plus the 85 associated with the oxidation of ascorbate). The maximum amount of CO$_2$ attained occured on Sol 13 and was approximately, 9,800 nmol. This decreased on Sol 15 to 9,400 nanomoles. No conclusion on the presence or change of CO can be made because of the low values of the combined Ar/CO peak. The values in Tables 3 and 4 of Ne and Kr demonstrate the consitency of the internal standards and the apparent precision for the gas analyses.

Wet mode results

On Sol 16, additional (estimated volume = 2.268 cm^3) M4 nutrient was injected. With the amount added earlier, the nutrient, now measuring 2.835 cm^3, wetted the soil. The results for the wet mode are shown in Table 4. The level of N$_2$ monotonically rose for an increase of 11 nmol whereas in the humid mode we had seen an increase followed by a decrease in the N$_2$ content.

Oxygen monotonically decreased from the plateau that was attained at the end of the humid mode and reached a minimum. The total amount of O$_2$ consumed equalised 325 nmol which is 78% of the ascorbate-oxygen equivalence added.

With water now making contact with the soil, the gaseous content of the interstitial water could be estimated. For the 1 cm^3 soil amount, an estimate of 5–50 µm particle sizes (personal communication from H. J. Moore, Viking Physical Properties Team) yielded an interstitial soil–water volume of about 35% (ref. 16). This value was used to estimate the total CO$_2$ in the solution contacting the soil, as separated from the CO$_2$ in the larger volume of water in the test cell sump that was treated separately as if it were unaffected by the soil. The interstitial soil solution and solution in the sump compartment shared a common headspace atmosphere from which the CO$_2$ was measured. The calculations show that CO$_2$ estimates are only meaningful to the extent that they represent a minimum.

During the entire first cycle, no H$_2$, NO or CH$_4$ was observed in the headspace.

Model for Martian surface chemistry

From very preliminary laboratory data (personal communication from Dr Edward Merek, Ames Research Center) using terrestrial volcanic ash, it was observed that some of the CO$_2$ adsorbed at Martian surface temperatures, 190–268 K, was desorbed at the higher incubation temperature of the test cell,

Table 3 Gas composition (corrected) in gas exchange test cell (humid mode)

	Time from humidification (h)				
	2.78	27.86	52.51	101.91	150.74
	Mars Date				
	Sol 9	Sol 10	Sol 11	Sol 13	Sol 15
Gas	Quantity of gas* (nmol)				
N$_2$	73	76	83	79	77
O$_2$	500	650	690	690	690
CO$_2$	5,900	8,300	9,500	9,800	9,400
Ar†	8	7	13	9	8
Ne‡	20	19	18	20	21
Kr§	2,000	2,000	2,000	2,000	2,000

*The gas chromatograph detector data were sampled at 1 s intervals, digitised and fitted to a skewed gaussian distribution from which peak heights were obtained. The gas in the headspace was obtained from the ratio of the sample loop volume to the total headspace. The cumulative gas composition was corrected for sampling losses by referencing absolute changes in the krypton values for successive samples. Corrections were made for pressure sensitivity in this flight instrument caused by a partial restriction in the gas sampling system which prevented total evacuation of the sample loop to ambient pressure before filling (three times) from the test cell. The volume for krypton was corrected for pressure as follows:

$$\text{nmol Kr} = 37.77\,(P_c)\exp\,-0.118\,(V_p)\exp\,1.016$$

where P_c is the test cell pressure in mbar, V_p is the peak height in volts. The value for each gas was corrected by the ratio of the term 37.77 $(P_c)\exp\,-0.118$ to the similar Kr value from a pressure-insensitive instrument. P_c was determined from an inventory of all sources of pressure such as the enclosed atmosphere, the pressure of the initialisation gas injected and any additional injections of helium required to increase cell pressure. The gas composition as stated was corrected by removal of contributions from known sources (for example trace contaminants in injected gases) and for the amount dissolved in the liquid phase. An estimate of dissolved gases was made from literature values for the Ostwald coefficients. The effects of pH on the CO$_2$ distribution were included by estimating changes in apparent CO$_2$ levels upon nutrient injections in LR (second injection) and upon the wet-mode nutrient injection in gas exchange. The relationship used was

$$\frac{\text{(nanomoles, dissolved)}}{\text{(nanomoles, gas phase)}} = L\,\frac{\text{(volume, liquid)}}{\text{(volume, gas)}}$$

where the L values for CO$_2$ are as follows. Sol 9 and 10: $L = 21.4$; Sol 11–15: $L = 28.4$; Sol 16: $L = 40.4$; Sol 17–28: $L = 68.5$. The data shown in this table and in Table 4 are revised from those given in ref. 1 by better values for the atmospheric composition and the composition of the krypton/carbon dioxide gas. The correction, subtracting these contributions from the total, tend to increase the values, but do not alter the conclusions reached.

†Assumed to be argon (argon is not resolved from carbon monoxide on this column).

‡Mean value for neon = 20.02±1.09 (±5.44%).

§Mean value for krypton = 1,975±0 (±0%).

281–283 K, during the first 21.23 h that the soil was sealed in the test cell. However, when the same volcanic ash was then humidified, major desorption of the CO_2 occured in 2.78 h. When the amount of CO_2 desorbed was related to the Martian soil, the 9,800 nmol of CO_2 reduced to 0.33 mg CO_2 desorbed per g soil. This value of desorbed CO_2 which is assumed to be adsorbed by Martian soil is within the model proposed by Fanale and Cannon[17] and supports the method we used to estimate total CO_2. The CO_2 production in the wet mode was probably due to oxidation of nutrient organic compounds.

Table 4 Gas composition (corrected) in gas exchange test call (wet mode)

	Time from 2.3 ml nutrient injection (h)					
	2.66	27.31	51.98	100.31	223.88	295.21
	Mars date					
	Sol 16	Sol 17	Sol 18	Sol 20	Sol 25	Sol 28
Gas	Quantity of gas (nmol)					
N_2	76	78	78	79	86	87
O_2	670	600	550	510	460	450
CO_2	9,600	11,000	12,000	13,000	13,000	12,000
Ar	8	8	7	8	9	8
Ne	100	150	160	170	170	180
Kr	2,000	2,000	2,000	2,000	2,000	2,000

See Table 3 for details of test and calculations.

The unexpected amount of O_2 accompanying the desorption of CO_2, 9,800 nmol/775 nmol (13 : 1 CO_2/O_2) from the minimum 270 : 1 CO_2/O_2 ratio in the Martian atmosphere[15] represents an oxygen enrichment of approximately 20 times in the Martian soil. It is improbable that the excess amounts of O_2 found beyond that expected from adsorption of atmospheric O_2 comes from the direct or biological photocatalysis of water or hydroxyl groups, for the reasons that the critical temperature for O_2 is too low for adsorption in the quantities needed and the diffusion rate of O_2 at 7.6 mbar is too rapid to allow for enrichment to occur. A more likely source for the O_2 is chemically bound oxygen which is stable at GEX cell temperatures but allows displacement of oxygen by water. The surface of the grains of rock or mineral are good sources for this chemically bound oxygen. A simple estimate of the surface area of the grains can be made to assess whether all of the oxygen can be accommodated. The surface area of the grains are assumed to be 400–2,000 cm^2 per cm^3 of soil (personal communication from H. J. Moore for 5–50-μm particle sizes). Assuming 33.4 $Å^2$ coverage per molecule, a monolayer of O_2 covers 1,560 cm^2, and is accommodated by the available grain surface. The requirement that chemical absorption cannot extend beyond a monomolecular layer[26] is pertinent.

A model of surficial materials which can accommodate both oxygen and CO_2 is solid silicate particles covered with fine iron oxide particles. The carbon dioxide desorbed from a monolayer would be accommodated on 19,710 cm^2 of the iron oxide surface (assuming the 33.4 $Å^2$ for CO_2 also). The iron oxide cover would be approximately three particles deep, under the conservative assumption that each iron oxide particle is a cube that has six times the surface area of the rock surface it covers. The least surface area is obviously a sphere having four cross sectional diameters of surface for each area of the mineral it would cover. If the mean particle size in the atmosphere (2 μm diameter) (ref. 19) is indicative of the iron oxide diameters on the surface, the mean thickness of the three-layer iron oxide is 6 μm. This is thick enough to make the inner surface opaque to the solar ultraviolet, but also too thick to be consistent with $\leqslant 2$ μm thickness reported by the Inorganic Chemical Analysis Team (ICAT) on the limits of iron oxide coating allowed for measurement of underlying silicate surfaces[18]. If the value of the particle sizes were closer to the lower conservative estimate of 0.2 μm (ref. 20), the mean thickness would be compatible with the ICAT limit of detection.

These estimates of surface area are preliminary best estimates and may be revised at a future date. Lunar fines[25] have surface areas near 5×10^3 cm^2 g^{-1} while clays[16] have surface areas in the range of 10^5–10^7 cm^2 g^{-1}. The results of the GEX may ultimately yield an estimate of the surface area of the Martian surface materials.

The mechanical structuring of the oxygen affixed to the inner mineral surface instead of on the outer iron oxide coat is consistent with known chemicals, alkali and alkaline earth metal peroxides and superoxides that are capable of producing both O_2 and H_2O_2 in the presence of water vapour[21]. A candidate for one of the metal superoxides is calcium because of calcium's apparent abundance in the Martian soil[18]. For each superoxide hydrolysed by water, an equivalent alkaline site is produced that accounts for the resorption of the desorbed CO_2. But in the natural state, the basicity of the inner surface is not transferred to the iron oxide coat, because the mineral surface is mechanically separated at the molecular level from the iron oxide coating of several layers. The H_2O_2 formed from superoxide hydrolysis will, in the presence of abundant iron oxide catalyst, directly participate in the oxidation of the exact number of oxidisable equivalents such as formate in the Labelled release (LR) nutrient[22] or GEX M4 nutrient[3]. The alkali and alkaline earth metal superoxides are only one of many examples of the proposed chemistry on a stoichiometric basis that can well be applied to the ozonides, perchlorates, permanganates, and so on.

The iron oxide external surfaces provide at low temperature a monomolecular layer of adsorbed CO_2 which when activated by the solar ultraviolet flux splits off nascent oxygen and CO. The latter is free to return to the atmosphere. At higher temperatures, ultraviolet catalysis is limited by the quantity of CO_2 adsorbed. Other sources of nascent oxygen are photochemically formed ozone and hydrogen peroxide which are catalytically decomposed by the surficial iron oxide to form O_2 and O and H_2O and O, respectively. Then ascent oxygen, by simple diffusion (albeit an inefficient process, but time is not of the essence) passes through the porous iron-oxide matrix and attacks the inner mineral surface where it reacts with alkali and alkaline earth metal oxides to form the hydroxy-peroxides, superoxides, peroxyradicals and/or ozonides, if it does not first recombine with CO or react with another nascent oxygen. On the inner mineral surface, the products of the nascent oxygen reaction remain relatively stable but are potentially vigorous oxidisers.

If the oxygen–soil model is correct, it implies that the oxidiser source is distributed generally throughout the entire soil sample bed, that is, through the entire depth of the trench dug on the first sample taken. On the other hand, if the activity were associated only with the surface, there would have to be ten times the activity from just the surface material. If the red colour is associated with this activity, the colour photographic data substantiate that the entire trench is the same as the surface (personal communication from T. A. Mutch, Viking Lander Imaging Team). Furthermore, only a small fraction of the actual surface material is probably represented in the soil sample acquired. For example, a simple test of the surface sampler at JPL on surface tinted soil shows that only 11% of sieve size material 1.5 mm $> d >$ 0.589 mm, 9% of 0.589 mm $> d >$ 0.495 mm and 3% of 0.495 mm $> d$ are of surface origin in the test sample. A distribution of the oxidative capability throughout the entire depth of the trench, not just located on the exposed surface, indicates a history of stability consistent with the concept of the protective iron oxide coat under which is buried the oxygen-producing source. The stability is contrary to having the oxygen coupled surficially on the iron oxide coat because it would be at the mercy of transient changes in the vaporous environment over extremely long periods of time.

Another aspect of the iron oxide coating which bears on the stability question is related to the weak acidic nature of this material. Qualities which are ascribed to acid-like surfaces are the following: the ready emission of CO_2 from the soil in GEX, the small first peak from the pyrolytic release experiment (PR), the initial instant release of $^{14}CO_2$ from the LR and the higher background counts when LR was cooled following heating of the test cell to sterilisation temperatures[1]. The significance of the LR sterilisation regime is that the temperature is sufficient to volatilise such materials as $H_2SO_4 \cdot 2H_2O$ (boiling point 169°C) which can coat surfaces and prevent readsorption of $^{14}CO_2$ at the LR test cell temperature. The latter observation provided us with a basis for speculating that surficial acidity is provided by a thin coat of sulphuric acid from sulphate salts of weak bases (amorphous sulphate). A probable source of this sulphate is SO_3 produced by the visible activity of the past[23]. The SO_3 reacts with any water vapour in the atmosphere to produce sulphuric acid that descends as a mist to cover the iron oxide coat. Such hygroscopic coatings could well preserve the integrity of the highly active inner superoxide coat during episodes of higher water vapour incidences on Mars while explaining durable O_2 production from the soil sample in the test cell. It may explain the observed cohesive nature of the Martian soil. The acid characteristic of the surface ascribed to amorphous sulphate is born out by the high level of sulphur measured by the ICAT (ref. 18) and the oxidised state of the surface as evidenced by the red colouration of the iron oxide outer coat. The acidic surficial characteristics are quickly neutralised by the strongly basic alkali and alkaline earth metal hydroxides formed by the hydrolytic reaction as demonstrated by both GEX and LR.

The above model of the silicate mineral covered with iron oxide requires that ultraviolet light initiate the process of nascent production where adsorbed CO_2 and/or H_2O is concentrated on the surface of the iron oxide particles. To maintain a steady-state concentration of these energy-rich oxygen bonds on the inner mineral surface, the combined dissipative effects of water vapour and heat must be counterbalanced by an equivalent influx of nascent oxygen. When temperatures are low and water vapour is virtually limited, as appears to be the case in the Lander 1 area, the oxygen sources are prevalent. Sites having more water vapour are expected to have less active material and produce less oxygen, but it remains to be determined whether the effective oxidative capacity, that is the capability to oxidise organics, is also reduced. The H_2O_2 product (unstable in the presence of iron oxide) may remain uncontacted by the iron-oxide outer coat.

The degree to which the particle surfaces are re-exposed to the ultraviolet flux to renew the activity, the penetration of nascent oxygen, hydrogen peroxide and/or ozone downward through the soil bed and the concentration of water vapour are factors that must be considered fully to describe and/or predict the potential to produce oxidisers at any site for any depth into the surface of Mars.

At the Viking Lander 1 site, the vista is an ancient one where the soil was formed before the boulders were strewn over it. This suggests that the soil was layered over fluvial scoured rocks well after the episodes of water formed the surface. The oxidisers could have been created by two distinctly different processes. During a period when there was a dense atmosphere, silicate particles could have been lifted into the atmosphere by winds allowing the surfaces to be exposed to solar ultraviolet thus creating oxidisers which have been preserved to this date. Oxidisers could also be formed constantly at a rate through the entire soil bed from atmospheric hydrogen peroxide, ozone or nascent oxygen providing a steady state production of oxidiser which counters the dissipative effects of warmth and humidity. Both ozone and hydrogen peroxide can provide nascent oxygen in our model.

The oxidisers in the Martian soil are good candidates for the reaction observed in the LR experiment[1] as well as some of the observed CO_2 evolution in GEX. They must have the requisite qualities of oxidising the formate in the LR, producing the O_2 in GEX and accounting for some of the CO_2 released in GEX. A reaction that could account for the above observations starts with 534 nmol of the metal superoxide, $M(O_2)_2$:

$$586H_2O + 534M(O_2)_2 \rightarrow 775O_2 + 534M(OH)_2 + 52H_2O_2$$

Thermodynamic calculations proving the validity of these types of reactions in the environment simulating GEX will be tested at a later date.

Thee quivalent amount of oxidiser in the LR (26 nmol per 0.5 ml soil) calculated by one of us (V.I.O.), indicates that the inferred H_2O_2 produced as a result of soil reacting with water is limiting.

Because of the large abundance of water with respect to formate in the LR (ref. 22), 55.56 M per 2.4×10^{-4} M $\sim 2.3 \times 10^5$ l^{-1}, and in the GEX 1.9×10^5 l^{-1}, it is unlikely that the superoxide will oxidise the formate directly, but will provide the requisite H_2O_2 in the reaction with water.

In the GEX, the N_2 changes are small and can be explained from either initial N_2 desorption from soil by water vapour and subsequent resorption in liquid water or biological fixation, while N_2 production could arise from a Van Slyke reaction where α-amino nitrogen in the M4 nutrient reacts on the aforementioned external-acid surfaces with nitrite in Martian soil. Since N_2 exists in the Martian atmosphere and there is postulated NO in the upper atmosphere[24], it would not be inconsistent to assume oxides of nitrogen exist in the Martian soil.

As we have observed from our studies of lunar soils[6,7], metallic iron formed by meteoric bombardment of lunar iron oxides provides a basis for estimating the biologically important, free-water history of a planet. At this time, no H_2 has been found, therefore no metallic iron can be inferred in the Martian sample tested by GEX. This suggests that an amount of water and/or O_2 necessary to oxidise the metallic iron to the depth of the regolith was available at some time for this oxidation. For answers on the question of the state of oxidation of the planetary surface as measured by the presence of metallic iron, we must await the recharge occurring at the start of the second cycle to remove O_2 so as to measure the production of H_2 from the oxidation of Fe° by H_2O, and await the findings of Lander 2. The ultimate sensitivity for H_2 detection will allow us to measure 0.003% of metallic iron in the Martian soil if O_2 is not present in the test cell.

The advantage of starting with metallic iron as a precursor source of iron oxide is that it presents iron in an easily oxidisable form that allows the propagation of the iron oxide product on a broad planetary scale without the need for liquid water. Only water vapour and O_2 are required. The early development of the iron oxide in the history of Mars explains how the dry lubricating qualities of the iron oxide dampens aeolian erosion and removes the need for liquid water to extrude iron oxide deposits from their sources.

We prepared earlier for an expected chemistry of an arid planet[3] and must await the dissipation before life can be determined. At this time, all of the gas changes we have observed in our GEX test cell can be explained most easily by mechanistic processes. Although these results and explanations are preliminary, we find no need to invoke biological processes. While continued careful study is required, it is anticipated that results from Viking Lander 2 will reinforce our conclusions.

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Storage of elastic strain energy in muscle and other tissues

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Storage of strain energy in elastic materials has important roles in mammal running, insect jumping and insect flight. The elastic materials involved include muscle in every case, but only in insect flight is the proportion of the energy stored in the muscle substantial.

MUSCLE has elastic properties and so do the tendons and skeletal elements to which muscles attach. These properties are exploited by animals in two main ways. First, energy is saved, in insect flight and in mammal running, by storing elastic strain energy at one stage in the wingbeat or stride and releasing it at another[1-3]. Second, some insects have catapult mechanisms which enable them to jump much higher than they otherwise could[4]. We have assessed the relative importance of the elastic properties of the muscles and of other structures in each of these activities. The arguments which follow show that the role of the elastic properties of the muscles is trivial in mammal running and insect jumping, but may be important in insect flight.

Capacity for elastic storage

Huxley and Simmons[5] investigated the elastic properties of active frog semitendinosus muscle. They compensated for the compliance of the tendons by a spot-follower device so that they measured only the compliance of the muscle fibres themselves. Their observations indicated that elastic extension occurs almost exclusively in the crossbridges. Each active crossbridge is stretched by 15 nm. The experiments of Jewell and Wilkie[6] with intact muscles indicated rather less stretching than this but those of Rack and Westbury[7] indicated that the crossbridges were stretched by about 30 nm.

Consider a muscle fibre of length l, sarcomere length λ exerting a force P. Let each crossbridge suffer an elastic extension b so that each sarcomere extends $2b$. The fibre as a whole extends $2bl/\lambda$ and the elastic strain energy U is $P/2$ times this

$$U = Pbl/\lambda \qquad (1)$$

Let the mass of the fibre be m and its density ρ so that its volume is m/ρ and its cross-sectional area $m/\rho l$. The stress σ in the fibre is given by

$$\sigma = P\rho l/m \qquad (2)$$

From equations (1) and (2) the strain energy per unit mass of muscle is

$$U/m = \sigma b/\lambda\rho \qquad (3)$$

Let the maximum stress which can be developed in isometric contraction be σ_0, and the corresponding strain energy U_0.

We have calculated values of U_0/m for various muscles, assuming that active crossbridges stretch 15–30 nm in the invertebrate muscles as well as in vertebrate striated muscle (Table 1). The values of U_0/m are all fairly similar because σ_0 is likely to be roughly proportional to λ[13].

The maximum work which can be done by a muscle in a single contraction is the area under a graph of isometric force against length. From Fig. 1 this can be shown to be 220 J kg^{-1} for frog striated muscle and 420 J kg^{-1} for a crayfish muscle with longer sarcomeres. Notice that these values are of the order of 100 times greater than the capacity of muscle for storing elastic strain energy.

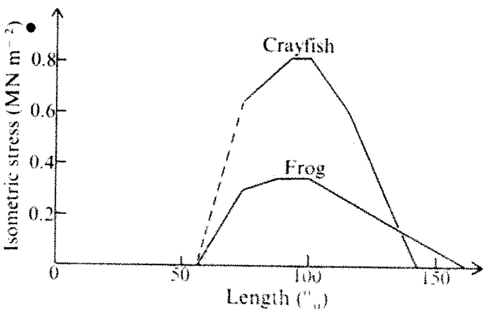

Fig. 1 Graphs of isometric stress against muscle fibre length for frog semitendinosus[8,15] and crayfish extensor carpopoditi[16]. Length is expressed as a percentage of the length at which the isometric stress is maximal.

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Now consider a muscle in series with a tendon of length l_t. When it exerts a force P it stretches the tendon by Δl_t and the elastic strain energy stored in the tendon is

$$U_t = \tfrac{1}{2}P\Delta l_t \qquad (4)$$

This can be expressed as a multiple of the elastic strain energy in the muscle, which is given by equation (1)

$$U_t/U = (\lambda/2b)(\Delta l_t/l) \qquad (5)$$

$\lambda/2b$ is 35–70 for vertebrate striated muscle and rather more for some insect muscles (Table 1). Tendons stretch about 10% (ref. 14) and insect apodemes about 3% before breaking[12]. Δl_t may thus be almost 0.1 l_t for tendons and 0.03 l_t for apodemes, if they are not much thicker than they have to be to withstand the forces their muscles exert on them. Hence U_t/U is likely to be greater than l_t/l. Pennate muscles with short fibres and long tendons or apodemes (that is, with large values of l_t/l) are likely to store far more elastic strain energy in their tendons or apodemes than in their muscle fibres.

Running

When a rubber ball bounces it loses kinetic energy and then regains most of it. The energy is stored briefly as elastic strain energy and converted back to kinetic energy in the rebound. Similarly a running mammal loses and regains kinetic and potential energy in every step. Some of this energy is stored as elastic strain energy in muscles and tendons and restored in an elastic recoil. The rest has to be replaced by metabolic work. Measurements of the oxygen consumption of running men and hopping kangaroos indicate that as much as 50% of the energy which would otherwise be needed for locomotion may be saved in each case by elastic storage[2,3].

Table 1 Strain energy stored in the series elastic component of various muscles in isometric contraction, calculated by equation (3) assuming a density of 1,060 kg m^{-3}

Muscle	Vertebrate striated	Locust flight	Hemipteran flight	Locust leg
Sarcomere length, λ (μm)	2.1	3.9	2.6	8.5
Maximum isometric stress, σ_0 (kN m^{-2})	350	350?	120	800
Strain energy U_0/m (J kg^{-1}) for:				
b = 15 nm	2.4	1.3	0.7	1.3
b = 30 nm	4.7	2.5	1.3	2.7
Reference	8	9	10, 11	12

The only leg muscles which can save energy in this way are the ones which first extend and then shorten while their foot is on the ground. The extensors of the ankle do this and so do the extensors of the knee (except in wallabies) but the other major leg muscles do not[3,17]. The extensors of the ankle and knee are pennate with long tendons, the Achilles and patellar tendons. Table 2 shows that far less elastic strain energy is stored in muscle fibres than in the Achilles tendon, in running men, hopping wallabies and jumping dogs. Strain energy has been calculated for the ankle and knee extensor muscles together, but for the Achilles tendon alone. We have not enough data to make reliable estimates of the elastic strain energy stored in the patellar tendon, but it is plainly substantial. The discrepancy between the quantities of strain enregy stored in the muscles and tendons must be even more marked than is apparent from Table 2.

The data for man is less reliable than the other data in Table 2. The value for muscle mass m was obtained from a dissecting-room cadaver (albeit an unusually robust one) and is almost certainly less than the mass of the corresponding muscles in the men who made the force-platform records. If so, the stress σ in the muscles will have been overestimated so the value of U (which is proportional to $m\sigma$, equation (3)) is probably about right.

Insects jumping

A boy using a catapult does work relatively slowly on the rubber, storing elastic strain energy which is released very rapidly in the elastic recoil. The power output of the catapult is thus much greater than the power input: the catapult serves as a power amplifier. Elastic structures are used in the same way by jumping insects. They are needed for the following reason.

Suppose an animal has muscles which, like vertebrate striated muscle, can do work amounting to 220 J kg^{-1} in a single contraction. Suppose that the jumping muscles make up 5% of the mass of the body. They are in principle capable of giving the body 11 J kinetic energy per kg body mass, that is of accelerating it to a take-off velocity of 4.7 m s^{-1} which is enough for a vertical jump in which the centre of mass rises 1.1 m. This is true, whatever the size of the animal. However, a small animal must accelerate to its take-off velocity over a very short distance, in a standing jump: it must give itself the kinetic energy in a very short time so the power output required (per unit mass of muscle) is high. Locusts take off at 3.2 m s^{-1} or less and the jumping muscles make up 7.5% of body mass so the work the muscles have to do is only 70 J kg^{-1}. However, the power required is 5,000 W per kg muscle, about ten times the maximum power output of the fastest known muscle[19]. Locusts and other jumping insects can only jump as well as they do because they have catapult mechanisms. Their leg muscles contract, storing elastic strain energy in themselves, their apodemes and other structures. This energy is released very rapidly at take-off, by a trigger mechanism.

Nearly all the energy for a locust jump is supplied by the extensor tibiae muscles. Table 1 shows that the elastic strain energy stored in these muscles before a jump is unlikely to be more than 2.5 J kg^{-1}, a very small fraction of the 70 J kg^{-1}

Table 2 Calculations of elastic strain energy in the muscles and in the Achilles tendon of one leg of a man running at 3.9 m s^{-1}, a wallaby (*Protemnodon rufogrisea*) hopping at 2.4 m s^{-1} and a dog taking off for a running long jump

	Man	Wallaby	Dog
Mass of animal (kg)	68	10.5	26
Mass of muscles, m (kg)	1.66	0.48	0.37
Muscle strain energy, U (J)	4.1–8.3	0.36–0.73	0.6–1.1
Force in tendon, P (N)	4,700	850	2,000
Extension of tendon, Δl_t (mm)	18	11	25
Tendon strain energy, U_t (J)	42	4.7	25
Reference	18	3	14

The muscles referred to are the extensors of the ankle and knee. The strain energy in them has been calculated from equation (3), using the stresses calculated in the references and the values of b and λ given in Table 1. The extensions of Achilles tendon in the wallaby and dog have been taken from references 3 and 14; the value for man has been calculated in the same way, assuming a cross-sectional area of 70 mm^2. The strain energy in the tendon has been calculated by equation (4).

which is needed. The rest is apparently stored as elastic strain energy in the apodemes of the muscles and in the semilunar processes of the knee joint[12].

Similar conclusions can be drawn from our knowledge of jumping by fleas and click beetles. The kinetic energy at take-off is around 20 J per kg muscle and 60–100 J per kg muscle for strong jumps by the flea *Spilopsyllus* and the beetle *Athous*, respectively[20,21]. Only a little of this can be stored as elastic strain energy in the muscles. Most of it is stored in other structures, in fleas mainly in pads of the elastic protein resilin.

Table 3 Assessment of the contribution of elastic strain energy in muscles to jumping by locusts and fleas

	Locust	Flea
Length of muscle fibre (mm)	4	0.3
Fractional shortening required, ε	0.23	0.11–0.20
Sarcomere length (μm)	8.5	5
Expected elastic strain, ε′	0.007	0.012
$\frac{\text{Muscle strain energy}}{\text{Total work}}$, $\frac{\varepsilon'}{\varepsilon}$	0.03	0.06–0.11
Reference	12	20,32

The geometry of the legs requires the muscle fibres to shorten by the fraction ε, to extend the legs at take off. If each crossbridge stretches by 30 nm the elastic strain of the muscle fibres will be ε′.

Table 3 confirms the conclusions of the past few paragraphs. It shows by a different argument that elastic strain energy in the muscles can contribute very little to jumping by locusts and fleas.

Insect flight

The role of elastic storage in insect flight is very like its role in running. Kinetic energy taken from the wings at the end of one stroke is stored as elastic strain energy and used in the next stroke. As much as 50% of the power which would otherwise be needed for flight may be saved by elastic storage[1,22].

The flight muscles of locusts in level flight deliver 170 W kg^{-1} (ref. 23). The frequency of the wing beat is 19 Hz so the work done by the muscles in each contraction is 9 J kg^{-1}. As much as 2.5 J kg^{-1} may be stored as elastic strain energy in their fibres (Table 1) depending on the stress which is developed. Larger quantities of elastic strain energy are stored in cuticular structures (mainly in structures made of resilin, ref. 24) but the contribution of muscle elasticity cannot be neglected. Many insects beat their wings at much higher frequencies but their muscles deliver little or no more power[22] so the work per cycle is much less and the possible contribution of muscle elasticity correspondingly more important. Many of them have myogenic muscles which shorten between 1% (in *Bombus*) and 3% (in *Calliphora*)[25]. In such muscles, the sarcomeres are around 2.5 μm long, so if the crosslinks can strain elastically by 30 nm, elastic strains of 2.2% are possible.

In oscillatory contraction these muscles exert greater forces during the shortening half cycle than during the lengthening half cycle. A graph of force against length (or of moment against wing angle, Fig. 2*a*) is a loop of which the area represents the work done in each cycle. The gradient of the long axis of the loop is the elastic stiffness of the muscle and approximately corresponds to the graph of force against length for the active muscle in the non-oscillatory condition.

The power output of such muscles is high. The output of flight muscles of the beetle *Oryctes* is 30 W kg^{-1} (at about 40 Hz) and for *Bombus* muscle (at a higher frequency) 60 W kg^{-1}, both measured *in vitro*[26]. Estimates based on the metabolic rate and the probable efficiency of muscle suggest that bees and flies may be able to produce muscle power outputs in the order of 200 W kg^{-1} (calculated from data of Weis-Fogh, in ref. 26). As the muscle seems to be able to store up to 1.3 J kg^{-1} (Table 1), it seems that at the quite moderate frequency—for a small insect with myogenic muscles—of 100 Hz, the muscle could store and release 130 W kg^{-1} which is of the same order as the active power output. The muscles should thus act as major stores to conserve the kinetic energy of the wings and as such should bring about harmonic oscillation of the wings.

It is unfortunate for this argument that simple harmonic oscillation of the wings rarely occurs (see ref. 27). It is usual to find that the angular velocity of the wing is fairly constant during both up and down strokes and that the wing direction changes abruptly at the extremes of the stoke[28,29]. This cannot be explained by the simple harmonic motion of an elastic oscillating flight motor.

One mechanism which causes this is the dipteran click mechanism[30]. The click mechanism in isolation acts in a manner similar to an electric light switch; at the two extremes of the travel, the mechanism is stable and the wing sets either top dead centre or bottom dead centre. When moved but a short distance from the extreme position, the wing when released snaps back to the extreme position but when moved beyond its midpoint, the wing continues to move until it clicks into the other extreme position. Depending on the geometry of the system, the mechanism can have a greater or lesser effect on the wing movement.

Figure 2*c* is a schematic graph showing the moment exerted by the click mechanism at different wing angles. Muscle elasticity tends to restore the wings to their mid position but the click mechanism acts in opposition to it, tending to send the wings to one or other extreme position. The click mechanism does not absorb energy (Fig. 2*c* shows a line, not a loop) but in the middle part of the stroke it stores elastic energy while the muscles are releasing elastic energy and vice versa. Figure 2*d* shows how the forces exerted by the click mechanism probably affect the other forces which act on the wing. They reduce or eliminate the inertia force in the middle section of the stroke so the wing accelerates early in the stroke, moves at fairly constant velocity in the middle part of the stroke and decelerates again at the end.

The click mechanism has other functions. It brings about the rapid torsional movements of pronation at the start of the downstroke and of supination at the bottom of the downstroke[27,30], which are utilised in the clap-fling and flip mechanisms of lift generation proposed by Weis-Fogh[22]. The energy

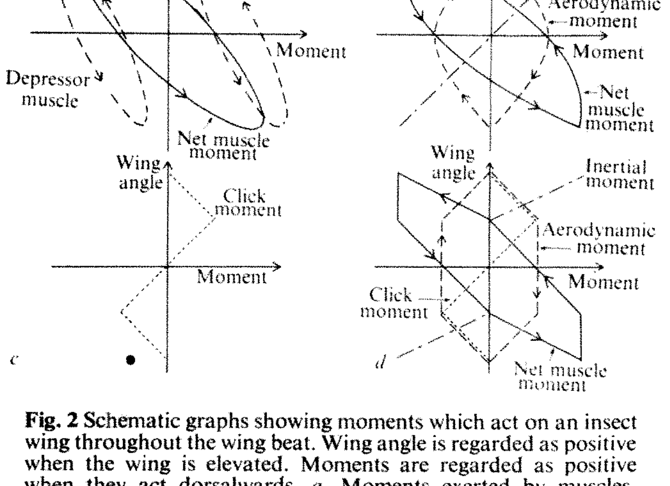

Fig. 2 Schematic graphs showing moments which act on an insect wing throughout the wing beat. Wing angle is regarded as positive when the wing is elevated. Moments are regarded as positive when they act dorsalwards. *a*, Moments exerted by muscles. The moments exerted by the levator and depressor muscles can be added together to give the net muscle moment. *b*, Moments on a wing which has no click mechanism. The moments shown must total zero at all stages in the wingbeat cycle. Inertial moment is shown proportional to wing angle, implying simple harmonic motion. *c*, Moments exerted by the dipteran click mechanism. *d*, Moments on a wing with a click mechanism. The moments shown must total zero at all stages in the wing beat. Readers will observe that the schematic graphs of net muscle moment in *a*, *b*, and *d* differ a little in shape. We do not know the precise shape of the loop for any intact insect and have obtained the shapes shown by making simple assumptions about the shapes of the graphs of other moments.

for these rapid movements is derived from the energy produced by the flight motor and the resultant kinetic energy of rotation of the wings, which is absorbed at the extremes of the wing stroke by the click mechanism and returned as rapid angular acceleration and torsional rotation of the wing. Thus the click mechanism acts as both a skeletal energy store and a power amplifier. Furthermore, since conventional cuticle can store 0.5–2 kJ kg^{-1} (ref. 31), the mass of the click mechanism may be as little as 1% of that of the flight muscles.

Role of series elastic component in locomotion

The series elastic component of muscle operates over small strains. Hence it can only store a small part of the work done by a muscle which shortens by a large fraction of its length. Also, it can only be responsible for a small part of the total store of elastic strain energy if the muscle is in series with a long compliant tendon. For these reasons it is relatively unimportant in insect jumping and mammal running. However, it is probably important in myogenic insect flight muscles, which operate over very small strains.

The quantity of elastic strain energy which can be stored in unit mass of material is 5 J kg^{-1} or less for muscle (Table 1) but it is 2,000–9,000 J kg^{-1} for tendon collagen, insect apodeme and resilin[4]. A structure for storing elastic strain energy can be much smaller if it is made of one of these latter materials than if it is made of muscle. The amount of work which can be done by a muscle in a single contraction may be 400 J kg^{-1} or more (Fig. 1) so a muscle designed to do a given amount of work will be far too small to store a similar amount of elastic strain energy. Hence the capacity of muscles for storing elastic strain energy is generally too small to be useful.

Insect flight muscles operate over very small strains so they do very little work in each contraction. However, they could not be replaced by smaller muscles working over larger strains because the power output required of them (around 200 W kg^{-1}) is apparently close to the maximum available from muscle[19]. Their capacity for elastic storage is large enough to be extremely useful. In general, the elastic strain energy to be stored in each cycle of wing movement is similar in quantity to the net work done by the muscles[22]. If the wing frequency is 20 Hz and the power output of the muscles 200 W kg^{-1}, the work done in each cycle is 10 J kg^{-1} and the capacity of the muscles for storing elastic strain energy may need supplementation. If the frequency is 200 Hz the work done per cycle (for the same power output) is only 1 J kg^{-1} and the capacity for storing strain energy may be ample even when partially counteracted by a click mechanism. Large insects such as locusts fly with low wingbeat frequencies and have structures such as relatively large resilin pads to supplement the elastic storage capacity of their muscles. Dipteran flies, being smaller, have higher wingbeat frequencies, and use click mechanisms which partly counteract the elastic compliance of the muscles. Their resilin hinges are relatively small.

Storage of elastic strain energy in muscles (or in tendons or apodemes in series with muscles) must imply an energy cost, since energy is needed to develop and maintain tension in muscle. We are unable to assess this cost for any of the activities we have discussed, but note that it will be least with slow muscles where storage is brief relative to the cycling time of the crossbridges.

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DNA base sequence of the $p_{\rm O}$ promoter region of phage λ

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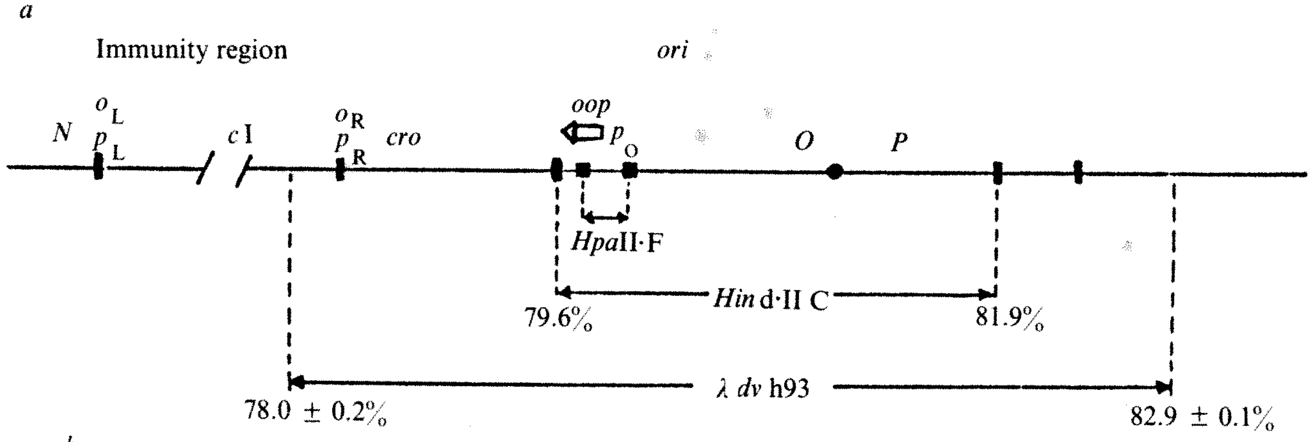
The sequence of a 123 base pair HpaII restriction fragment of bacteriophage λ DNA has been determined by the dimethylsulphate–hydrazine technique. Of this fragment 65 nucleotide pairs are transcribed into the 5′ proximal part of the oop RNA. The remaining 58 nucleotide pairs preceding the start point of transcription show homologies to the $\lambda p_{\rm L}$ *and* $\lambda p_{\rm R}$ *promoter regions, and are concluded to contain the* $\lambda p_{\rm O}$ *promoter sequence.*

REPLICATION of bacteriophage λ DNA proceeds bidirectionally[1] starting from a well defined region positioned between 79.9 and 80.6% on the λ physical map[2]. All known *cis*-dominant mutants affecting the origin of replication (*ori*) map within this region[3-6]. A small RNA molecule of known sequence[7] is transcribed from the *l* strand of this DNA segment (79.9–81.1) (ref. 8), and there is evidence that this RNA acts as a primer in the initiation of λ DNA replication[9]. Both the synthesis of this *oop* RNA, and the initiation of λ DNA replication require the products of the phage genes *O* and *P* as well as the *dna*B and the *dna*G gene products of the host[9,10]. In addition, mRNA synthesis initiated in this area has been shown to be involved in transcription leading to repressor establishment during lysogenisation[11]. This suggests that the *oop* RNA may be equivalent to a mRNA leader transcript. Because of this role in lysogenisation, phage gene *c*II and *c*III products may also modify initiation or attenuation[12] rates of the *oop* RNA synthesis.

As a step towards elucidating the structural features of this area in more detail we have undertaken to sequence the promoter region of the *oop* RNA. The 81-nucleotide sequence of the *oop* RNA as reported in ref. 7 contains a CCGG sequence at positions 68–71, which indicates a restriction enzyme *Hpa*II sensitive site[13] for the corresponding DNA sequence. Therefore, the generation of a restriction fragment of at least 70 base pairs could be expected with this enzyme. This fragment would represent the DNA complement of most of the *oop* RNA sequence and perhaps part or all of the p_O promoter region. In order to deal with a smaller number of *Hpa*II restriction fragments, we have used the small λ-derived plasmid λ*dv*h 93 (ref. 14) instead of DNA from the whole λ phage (see Fig. 1). Cleavage of λ*dv*h 93 DNA with *Hpa*II enzyme produces 10 major fragments, 7 of which have more than 70 base pairs (A to G, Fig. 1). For further selection among those *Hpa*II fragments, the fragment λ*dv*h 93–*Hin*dII·C (ref. 14) was isolated, since it spans the region where the *oop* RNA has been mapped (Fig. 1). Only four of the fragments with more than 70 base pairs were produced on digestion with enzyme *Hpa*II: fragments B, C, E and F (Fig. 1). Fragment T, which is not observed in the *Hpa*II digest of total λ*dv*h DNA, comprises the terminal portion of the *Hin*dII·C fragment that contains the terminator region of the *oop* RNA (E. Schwarz, unpublished work from this laboratory, and ref. 15). The decanucleotide d(T–G–G–A–T–C–T–A–T–C) whose sequence is complementary to the nucleotides 4–13 of the *oop* RNA sequence (see Fig. 4) had been synthesised chemically in our laboratory (D. Ghosal, unpublished). In a reaction catalysed by DNA polymerase I with linearised and denatured λ*dv*h 93 DNA as a template, this decanucleotide acts as a primer near the p_O promoter region. When the isolated and denatured *Hpa*II fragments B, C, E and F were tested in four parallel experiments as templates in such reactions, only the fragment *Hpa*II·F gave primer-dependent products (D. Ghosal, in preparation).

Sequence determination of the fragment *Hpa*II·F

For determination of the DNA sequence of the restriction fragment by use of the dimethylsulphate/hydrazine technique developed by W. Gilbert and A. Maxam, the fragment *Hpa*II F was first labelled at its 5′ ends using polynucleotide kinase and [λ-^{32}P]-ATP. As no internal restriction enzyme sensitive site was available for separating the two labelled 5′ ends, strand separation by electrophoresis on a 10% polyacrylamide gel[16] had to be performed (Fig. 2). In order to reduce cross contamination of the two strands, both were purified further by a second strand separation cycle. The dimethylsulphate–hydrazine technique (W. Gilbert and A. Maxam, personal communication, and ref. 17) consists of four parallel reactions, two of them being purine specific, with dimethylsulphate as a reagent, the other two being pyrimidine specific, using hydrazine. The products of the four limited (one random hit per chain) degradation reactions are fractionated according to chain lengths in adjacent slots on a denaturing polyacrylamide gel. The patterns of radioactive products are then made visible by autoradiography. The methylation conditions for the G and A specific reactions are identical, but the release of the methylated bases is different: neutral release at higher temperature (15 min at 90 °C) is more specific for the methylated G sites and results in a final gel pattern with heavy bands representing polynucleotides terminating at the G positions; only light bands are obtained from the A positions ('G-specific' reactions). Acid release (60 min at 0 °C in 0.1 N HCl) gives the opposite result, dark bands for every A position and light bands for every G position



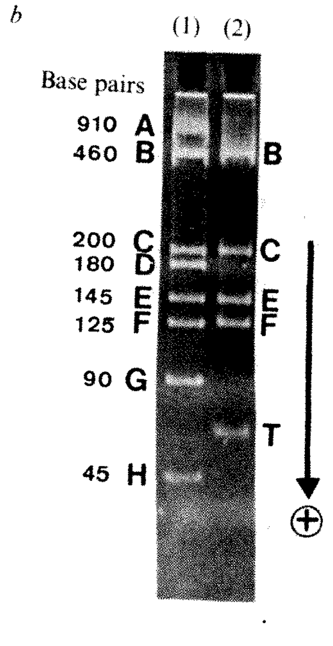


Fig. 1*a*, Physical and genetic map of the immunity and *ori* regions of phage λ. The position of the *Eco*RI cut (●) at 81.0% of the λ physical map[26] and of several *Hin*dII sites (▮)[14] are indicated, as well as the location of only two of the *Hpa*II cuts (■) generating the *Hpa*II·F fragment used in this work. The position of the small λ-derived plasmid λ*dv*h 93 (molecular weight 1.45 × 10^6) and of the *Hin*dII·C fragment are taken from ref. 14. For the designation of genes see ref. 27. p_O symbolises the promoter region for *oop* RNA synthesis. The map is drawn to scale. *b*, DNA fragment patterns obtained from *Hpa*II digests of λ*dv*h 93 DNA (1) and of the *Hin*dII·C fragment (2). The *Hpa*II (ref. 28) and *Hin*dII enzymes (ref. 29) were purified according to the methods of Smith and Wilcox[29]. λ*dv*h 93 DNA was prepared as described previously[14] and the *Hin*dII·C fragment was obtained by separating a *Hin*dII digest of λ*dv*h 93 DNA on a preparative 2% Agarose slab gel. The respective gel region was excised, crushed and extracted in NTE buffer (0.5 M NaCl, 0.1 M Tris-HCl, *p*H 8.0, 5 mM EDTA) at 30 °C for 2 d. Gel fragments were then removed by filtration through glass wool, and the extracted DNA was ethanol precipitated (2 volumes, 15 min at −70 °C), centrifuged at 10,000*g* for 20 min and resuspended in 1 mM Tris-HCl, *p*H 8.0. For the experiments shown in wells (1) and (2), incubations were carried out in 6.6 mM Tris-HCl, *p*H 7.5, 6.6 mM $MgCl_2$, 6.6 mM β-mercaptoethanol at 37 °C for 4 h and the material was then subjected to electrophoresis on a 10% polyacrylamide slab gel (20 cm × 30 cm × 0.2 cm) run in TBE-buffer (50 mM Tris borate, *p*H 8.3, 1 mM EDTA)[30]. After running the gel at room temperature for 13 h at 150 V, the bands were made visible by soaking the gel for 15 min in TBE-buffer containing 2 μg ethidium bromide ml^{-1}. The band patterns were then photographed under ultraviolet light. The numbers adjacent to the letters indicate the estimated numbers of base pairs of the respective fragments. The estimations were done by comparison with known restriction fragments of λ*dv* DNA (ref. 14).

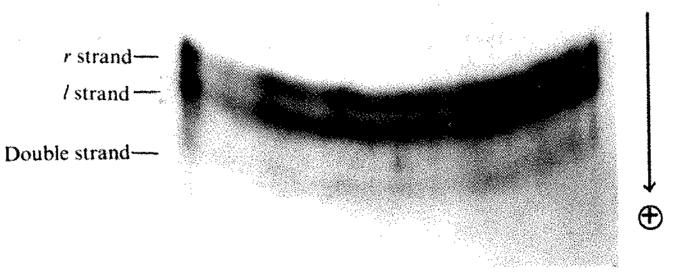


Fig. 2 DNA strand separation of fragment *Hpa*II·F. Preparative amounts of the restriction fragment *Hpa*II·F were obtained by cleaving 300 µg of λ*dv*h 93 DNA with enzyme *Hpa*II, separating the products on a preparative 10% polyacrylamide slab gel (20cm × 30 cm × 0.4cm) and extracting the fragment as described in Fig. 1. For the 5′-terminal labelling, about 10 pmol of fragment *Hpa*II·F were incubated with 0.2 units of alkaline phosphatase from calf intestine (grade I, Boehringer-Mannheim Biochemicals) in 10 mM Tris-HCl, *p*H 9.3 at 37 °C in a total volume of 40 µl. The reaction was stopped after 30 min by the addition of 8 µl of 50 mM EGTA and boiling the whole mixture in a sealed capillary for 3 min. 200 pmol of [γ^{32}-P]-ATP (prepared by the method given in ref. 31 with a specific activity of about 1,000 Ci mmol^{-1}) and 5–8 units of polynucleotide kinase (Boehringer-Mannheim Biochemicals) were added with 8 µl 10 × kinase buffer (0.4 M Tris-HCl, *p*H 9.3, 0.1 M $MgCl_2$, 10 mM DTE, 50% glycerol) and the reaction mixture (80 µl) was incubated for 30 min at 37 °C. After terminating the reaction with 10 µl of 0.5 M EDTA, the mixture was adjusted to 0.3 M sodium acetate. Ethanol precipitation was then carried out in the presence of 100 µg of carrier tRNA (2 volumes ethanol, 15 min at −70 °C). The dried pellet was resuspended in 300 µl of strand separation solution (10% glycerol, 0.3 N NaOH, 1 mM EDTA) according to W. Gilbert and A. Maxam (personal communication) and loaded on a 10% polyacrylamide slab gel in a sample well of 8.5 cm × 0.4 cm. The gel was run in TBE-buffer at 4 °C and 300 V, until the xylene cyanol FF marker applied in a separate well had reached the bottom of the gel (30 cm). X-ray film (Agfa Gevaert Osray T4) was exposed for 30 min to the gel covered with polyethylene wrap. Under the conditions given, the *r* and *l* strands (at 20.0 and 20.8 cm from the origin) were clearly separated. The small band seen below the separated strands represents undenatured (or renatured) double-stranded DNA. The regions containing the separated strands were cut out and extracted twice with 2 ml each of NTE buffer at 30 °C for two 24 h periods; this led to about 90% recovery of radioactivity. On running a second strand separation cycle (not shown) the final yields ranged between 1 × 10^5–2 × 10^5 c.p.m. per strand, as measured by Čerenkov radiation.

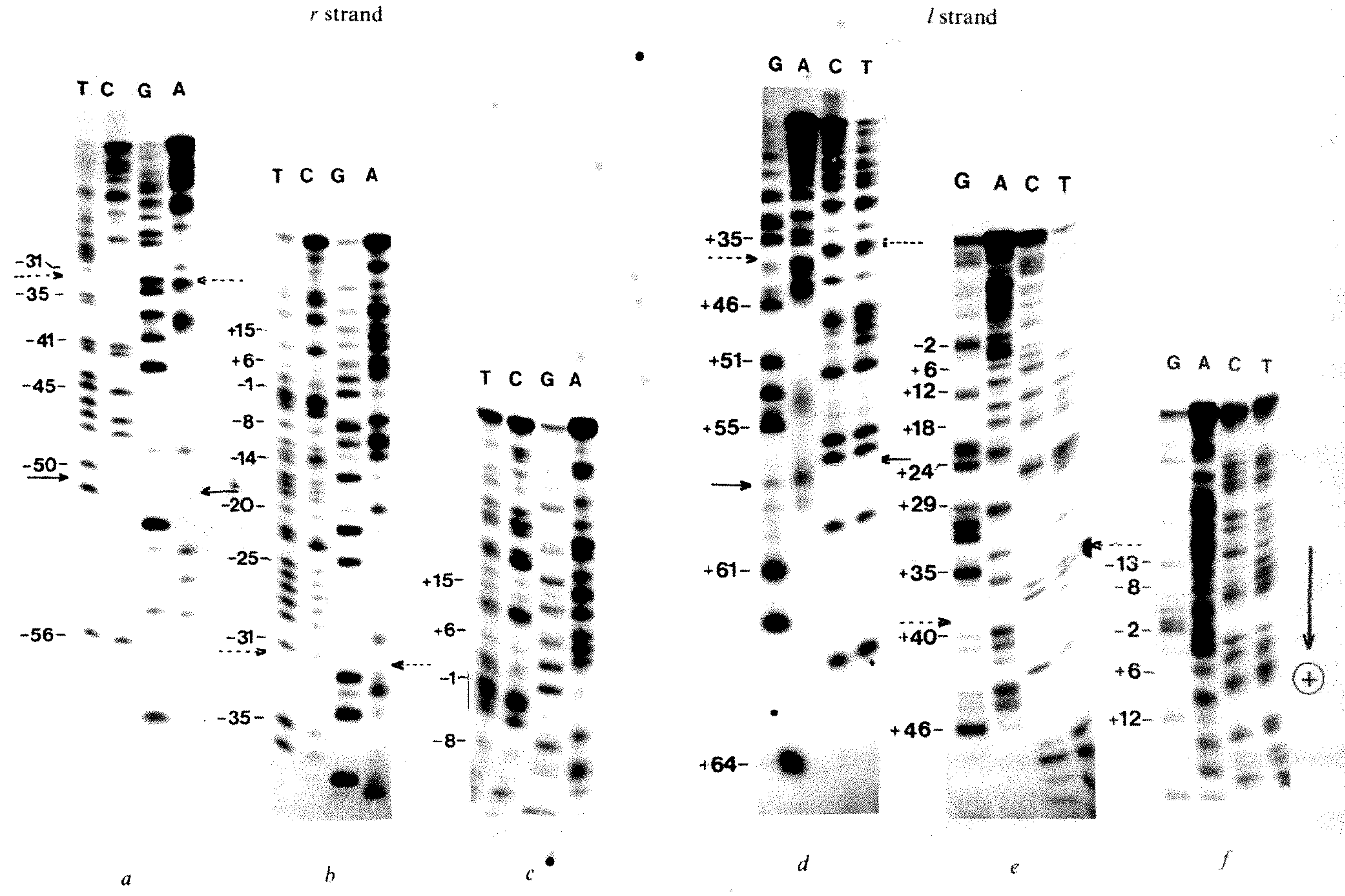


Fig. 3 Determination of the sequence of the *r* strand and the *l* strand of fragment *Hpa*II·F with the dimethylsulphate–hydrazine technique. 80 µg of sonicated calf thymus DNA were added to the separated strands, isolated as described in Fig. 2. After ethanol precipitation the material (about 1 × 10^5 Čerenkov counts) was divided into four equal parts. The two dimethylsulphate reactions (G and A specific; 30 °C and 37 °C respectively) were carried out in 500 µl of 50 mM sodium cacodylate, *p*H 8.0, 10 mM $MgCl_2$, 0.1 mM EDTA containing 1–2 µl of 10.7 M dimethylsulphate; the two hydrazine reactions were carried out at 37 °C in volumes of 50 µl (15 M hydrazine for the T-specific and 15 M hydrazine, 1 M NaCl for the C-specific reaction). The work-up of the reaction mixtures followed essentially the protocol of W. Gilbert and A. Maxam (personal communication and ref. 17). Incubation times were 60 min for the experiments shown in (*a*) and (*d*), 45 min for (*b*) and (*e*) and 30 min for (*c*) and (*f*). The cleavage products were finally fractionated on a 20% denaturating polyacrylamide slab gel (20 cm × 40 cm × 0.2 cm) containing TBE-buffer and 7 M urea. Electrophoresis was performed at room temperature applying 450 V for 17 h (*a*) and (*d*), 500 V for 42 h (*b*), 600 V for 21 h (*e*) and 600 V for 61 h (*c*) and (*f*). For the experiments shown in (*a*) and (*d*), a prerun was made at 600 V for 4 h. After electrophoresis the wet gels were covered with polyethylene wrap and exposed to X-ray film at −20 °C for 6–10 d. The numbers besides the autoradiograms refer to the corresponding positions of the *Hpa*II·F sequences shown in Fig. 4. The solid and dashed arrows give the position of the brompohenol blue and of the xylene cyanol FF markers, respectively. The columns labelled, T, C, G and A show the products of the T-, C-, G- and A-specific reactions, respectively. The heavy bands on top of each column represent unreacted starting material.

('A-specific' reaction). Limited reaction with hydrazine and no salt leads to a final product pattern with equal intensities for every T and C position ('T-specific' reaction), while the addition of 1 M sodium chloride to the hydrazine reaction mixture results in dark bands for the C positions and in light bands for the T positions ('C-specific' reaction).

Figure 3 shows the product patterns obtained by this technique for the separated *r* and *l* strands of the DNA fragment *Hpa*II·F. Three autoradiograms taken from three different electrophoresis gels are shown for each strand. They add up to more than 70 nucleotides for each strand. The final sequence as derived from both complementary strands is given in Fig. 4.

The following comments about the autoradiograms should be noted.

(1) The enzyme *Hpa*II recognises the double-stranded equivalent of the sequence 5′-CCGG-3′ cutting after the first C to produce restriction fragments starting with CGG at the 5′ ends[13]. However, the very first nucleotides of the chains could not be resolved, and the lowermost bands clearly visible in Fig. 3*a* and *d* indeed correspond to the third nucleotides from the 5′ ends (nucleotide numbers −57 and +64, respectively). This can be deduced from the bromphenol blue marker, which indicates the position of fragments 10 nucleotides long and by comparing the sequence derived from the *l* strand with the overlapping portion of the DNA sequence reported in ref. 15.

(2) There are two positions in the autoradiograms shown, where the G-specific reaction gives dubious results: the G at position +11 (*l* strand) and the A at position −10 (*r* strand). In the first case, the G-specific reaction gives a rather weak band compared with the G band corresponding to position +12, while in the second case a rather strong band appears at an A-position (but in the G-specific reaction). These irregularities have proved to be reproducible in several experiments. The ambiguities could be resolved, however, by looking at the corresponding positions of the complementary strand sequence. Further independent proof for the GG sequence in position +11/+12 (*r* strand) comes from the fact that the synthetic decanucleotide d(T-G-G-A-T-C-T-A-T-C) containing this GG doublet can act as primer in this region (position +13 to +4) (D. Ghosal, unpublished work from this laboratory).

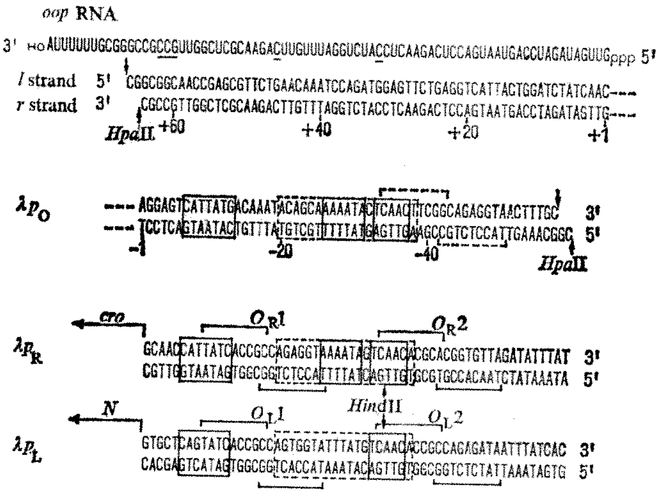
(3) The CTCCT sequence at positions −5 to −1 in the *r* strand is not clearly resolved, but by inspecting the corresponding product pattern derived from the *l* strand, the sequence AGGAG (−1 to −5) can clearly be seen.

(4) Occasionally 'ghost' bands can be detected; these are due to small cross contaminations by the complementary strand. They can easily be assigned to the complementary strand, if the products of the four reactions of both strands are fractionated side by side on the same gel. As an example, the three ghost bands at positions −48, −46, and −44 in the G-specific reaction of the *r* strand (Fig. 3*a*) represent the G positions +55, +53, and +51 of the contaminating *l* strand (Fig. 3*d*).

Sequence features of the *oop* DNA and of the p_O promoter region

The sequence of the fragment *Hpa*II·F as resulting from the autoradiograms shown in Fig. 3 is presented in Fig. 4 in two halves; the upper part shows the section (*oop* DNA) transcribed into *oop* RNA; the lower part represents the section not being transcribed including the p_O promoter region. For comparison, the sequence of the λ promoters p_R and p_L as taken from ref. 18 are included in this figure. The segment including positions +53 to +66 is identical to the corresponding part of a sequence reported in ref. 15. (From this overlap the position of fragment *Hpa*II·F on the physical map of λ, as shown in Fig. 1, could be deduced.) From the DNA sequence determined we predict a sequence for the *oop* RNA (written as the top line in Fig. 4), which deviates from the reported sequence[7] at three positions (underlined in Fig. 4) as follows.

(1) According to the present data the triplet GCC has to be inserted at the positions 60 to 62, which is in accordance with this part of the sequence given in ref. 15. (2) At position 46 a single C residue replaces an octanucleotide GCCGCCUU of the reported sequence (positions 46 to 53 of ref. 7). (3) Finally there are two C residues proximal to the AU dinucleotide at positions 33–34, and only one C residue distal to it, instead of the reverse.



oop RNA
3′ HOAUUUUUUGCGGGCCGCCGUUGGCUCGCAAGACUUGUUUAGGUCUACCUCAAGACUCCAGUAAUGACCUAGAUAGUUGppp 5′
l strand 5′ CGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAAC---
r strand 3′ CGCCGTTGGCTCGCAAGACTTGTTTAGGTCTACCTCAAGACTCCAGTAATGACCTAGATAGTTG---
*Hpa*II
+60
+40
+20
+1
λp_O
---AGGAGTCATTATGACAAATACAGCAAAAATACTCAACTTCGGCAGAGGTAACTTTGC 3′
---TCCTCAGTAATACTGTTTATGTCGTTTTTATGAGTTGAAGCCGTCTCCATTGAAACGGC 5′
−1
−20
−40
*Hpa*II
cro
O_R1
O_R2
λp_R
GCAACCATTATCACCGCCAGAGGTAAAATAGTCAACACGCACGGTGTTAGATATTTAT 3′
CGTTGGTAATAGTGGCGGTCTCCATTTTATCAGTTGTGCGTGCCACAATCTATAAATA 5′
N
O_L1
*Hind*II
O_L2
λp_L
GTGCTCAGTATCACCGCCAGTGGTATTTATGTCAACACCGCCAGAGATAATTTATCAC 3′
CACGAGTCATAGTGGCGGTCACCATAAATACAGTTGTGGCGGTCTCTATTAAATAGTG 5′

Fig. 4 Comparison of the sequence of the promoter p_O with the promoters p_R and p_L of phage λ. The sequences of the p_R and p_L promoters were taken from refs 18 and 32. The sequence predicted for the *oop* RNA is written on top of the transcribed section of the *Hpa*II F fragment; at the positions underlined the shown sequence deviates from the RNA sequence reported in ref. 7, as discussed in the text. The start points of transcription of the genes adjacent to p_L and p_R are indicated by horizontal arrows at the left ends. The boxes at left show the RNA polymerase binding sequences[21] of the three promoter regions. The solid and dashed boxes further to the right point out sequences of total or partial (AT versus GC) homology between the three promoter regions. The presumed repressor binding sites in the p_R and p_L promoter regions[18,32] are indicated by solid brackets, a similar sequence in the p_O promoter region by dashed brackets. The positions of the *Hind*II sites in p_R and p_L are also shown. The p_R promoter is given as the inverse of its usual orientation in λ DNA.

This latter change is in agreement with the absence of an *Eco*RII cut from the *oop* DNA region (DNA pregrown under *mec*$^-$ conditions[19]; unpublished observation from this laboratory). The CCUGG of the original sequence, which is the RNA analogue to one of the two recognition sequences of the *Eco*RII restriction enzyme[20], is now replaced by the sequence UCUGG in positions 34 to 38 (Fig. 4). As a consequence of the revisions given here, the entire sequence of the *oop* RNA is now predicted to consist of 77 rather than of 81 nucleotides.

In the promoter model put forward by D. Pribnow[21], a promoter comprises three essential elements covering about 40 base pairs of DNA: a 'recognition' sequence, a 'binding' sequence, and an RNA initiation point. Five or six base pairs separate the binding sequence from the initiation point and a distance of about 20 base pairs is postulated between the binding and the recognition sequences. All the nine promoters the sequences of which have been published (see refs 21 and 22) show strong homology in a seven base pairs region which is believed to define the binding sequence[21,23]. The p_O promoter region presented in this paper fully confirms this rule as indicated by the boxed sequence of Fig. 4, six base pairs ahead of the start point of transcription. While the binding sequence has now been well accepted, there is still uncertainty about the basic structure of the RNA polymerase recognition site. Comparison of the λp_R and λp_L promoters with a promoter for *E. coli* RNA polymerase in SV40 DNA (ref. 18) and of the G2 and G3 promoters of phage fd DNA with the same SV40 promoter[22] revealed a five base-pair homology between these promoter sequences in the regions of the presumed recognition sites. The sequence of the p_O promoter region presented in Fig. 4 substantiates these findings. If one aligns these promoter sequences relative to their common binding sequences (and not to their RNA initiation points), a homologous hexanucleotide sequence emerges:

λp_{O}	5′ AGTTGA 3′
λp_{R}	TGTTGA
λp_{L}	TGTTGA
SV40	TGTTGT
fdG2	TGATGC
fdG3	CGTTGT

This sequence is placed always 20–25 nucleotide pairs ahead of the binding sequence (19–24 in the case of the fdG3 promoter). As has already been noticed[21,22] the *lac* and the *tyrosine tRNA* promoter sequences do not fit into this group. Obviously, more sequence data are necessary in order to identify possible additional groups of basic recognition sequences. (Due to a different adjacent nucleotide (Fig. 4) the p_{O} promoter DNA is just missing to also contain a *Hin*dII site (GTPyPuAC) as do the promoters λp_{R} and λp_{L} in this area.)

The λ promoters p_{R} and p_{L} overlap with the serial operator sequences in O_{R} and O_{L} (refs 18 and 32). Whereas the structural features of $O_{\mathrm{R}}1$ (or $O_{\mathrm{L}}1$) seem to be absent from the p_{O} promoter sequence, an operator-like sequence can be detected in the p_{O} promoter region, which is in exact register with the $O_{\mathrm{R}}2$ and $O_{\mathrm{L}}2$ operators (indicated by dashed brackets in Fig. 4). This sequence could be expected to act as a repressor binding site, but no reduction of the *oop* RNA transcription rate upon the presence of repressor has been observed *in vivo*[8]. In addition, no binding other than to the O_{R} and O_{L} fragments has been observed with λ*c*I repressor and *Hpa*II digestion mixtures of λDNA (ref. 24) and λ*dv*h93 DNA *in vitro* (unpublished observation from this laboratory). Since binding sites for the *cro* gene product in O_{L} and O_{R} apparently overlap with *c*I repressor binding sites, an alternative explanation for the operator-like sequence in the p_{O} promoter region would be to function as an interaction site for the *cro* protein. This possibility remains to be tested directly, although several mutations of phage λ have been mapped in this part of the *ori* region which show an increased sensitivity against *cro* (ref. 25). To identify other possible significant sequence structures, work is in progress to extend the known part of the p_{O} promoter region.

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letters to nature

X-ray flare in 3U 0833−45 the Vela pulsar

DURING an extended observation with the Ariel V Sky Survey Instrument (SSI) a flare has been detected from the X-ray source 3U 0833−45, generally associated with the Vela pulsar. Details of the SSI, which covers the energy range 2.4–18 keV, are given elsewhere[1]. The flare occurred during the first part of 24 July 1976, reaching a peak intensity at ~0300UT of 11.9 ± 1.7 counts s^{-1}, a factor of three above its normal (Ariel V) level, and lasting ~0.5 d. Figure 1 shows the light curve of the event, and it is interesting to note a possible precursor to the main flare, occurring approximately 2 d earlier. No similar event or measurable variability has been seen in observations of 3U 0833−45 with the SSI over the previous 20 months. A typical run of data from August 1975 with 3U 0833−45 in its normal (quiescent) state is shown in Fig. 2. These lower flux values have been summed in 0.7 d bins for clarity in the figure.

3U 0833−45 is believed to be associated with the pulsar PSR 0833−45 (ref. 2) itself probably a remanent of the Vela SN explosion[3]. Pulsed emission at both hard[4] and soft[5] X-ray energies have been claimed for this pulsar, although, as yet, efforts to confirm these results have proved unsuccessful[6–8]. It appears that the earlier results were incorrect or that the pulsed component is highly variable.

The chance of an unknown field source appearing briefly in the $0.7° \times 10°$ (FWHM) collimator of the SSI and coincident with the Vela pulsar in the scanning direction (offset $= 0.1° \pm 0.2°$) cannot entirely be ruled out. However, consideration of the frequency of such transient events found by Ariel V makes this very unlikely. Accepting, then, that the observed flare is indeed from the pulsar and taking a distance of 460 pc[10] the total emitted energy (2–10 keV), assuming the spectral shape of Kellogg *et al.*[2], of the main and precursor flares is $2.9 \pm 0.3 \times 10^{38}$ erg and $1.2 \pm 0.3 \times 10^{38}$ erg respectively.

Mapping of the Vela region with Ariel V (A. Smith, unpublished) has confirmed the result first obtained from an analysis

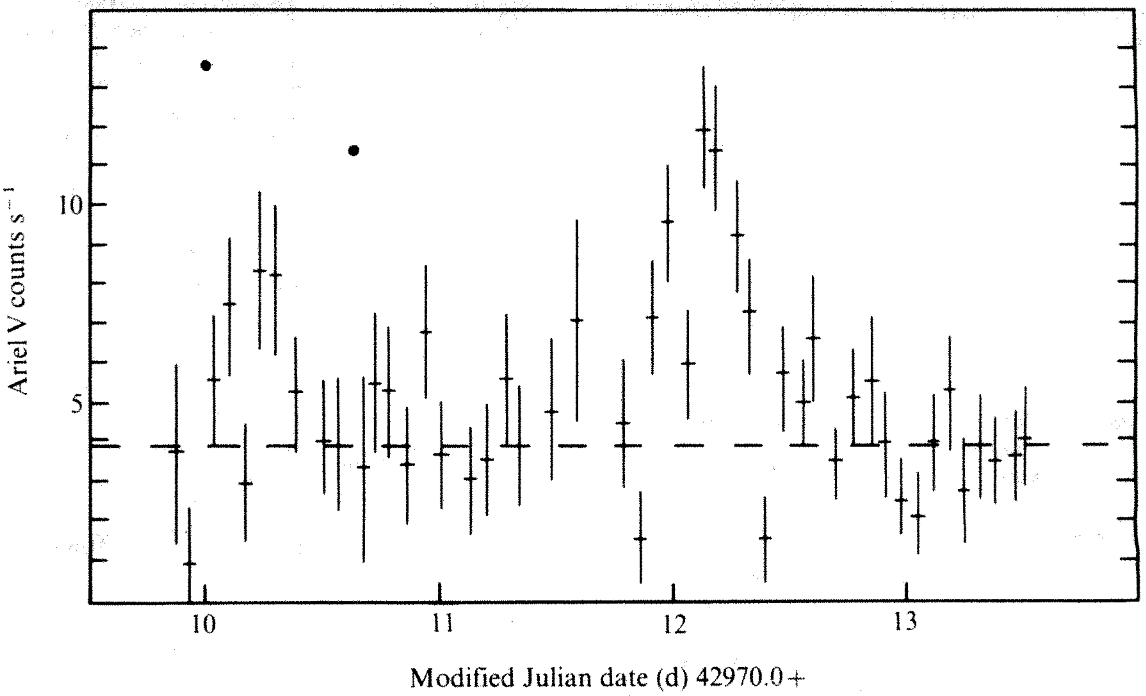

Fig. 1 Single orbit observations of 3U0833−45 during July 22–25 1976. Error bars are $\pm 1\sigma$. The broken horizontal line represents the normal flux for this source.

of Copernicus X-ray data by Silk[11], that the emission region around the Vela pulsar is extended. This extension is consistent with the normally steady X-ray emission of 3U 0833−45 and it is probable that the variable component now associated with the pulsar is generally undetectable and, correspondingly, that the variability of this point source component is much greater than a factor of three. This conclusion offers the possibility that the large pulsed component found by Harnden and Gorenstein[4] was indeed correct and occurred during a period of enhanced emission. The likelihood of a short rocket observation coinciding with such a flare can be estimated from the previous SSI data, some 600 orbits of which spread over 20 months detected no similar flares. The estimated probability of a flare coinciding with one of six reported observations is < 10%.

The 0.5 d timescale of the present X-ray flare may give a clue to its origin. Unfortunately no timing data was available during the observations and therefore it is not known whether the X rays were pulsed at the 89 ms period of PSR 0833−45. However, the absence of a concurrent change in the radio frequency ($\Delta\omega < 5\times10^{-10}\omega$, Manchester, personal communication) argues against the pulsar being the source of the flare. Equally, the short time scale rules out a major change in the X-ray emission of the extended nebular source (diameter of 30 arc min $\simeq$ 12 light years at 460 pc). The most probable source of the X-ray flare appears to be a redistribution of the magnetic field and particle flux in the magnetosphere of the pulsar. Scargle and Pacini[12] have discussed this possibility for the Crab Nebula to explain the occurrence of pulsar 'spin-ups' and 'spin-downs' and the considerably greater energy associated with the concurrent wisp activity.

The energy stored in the magnetosphere of PSR 0833−45 may be of the order of the magnetic energy of the neutron star: $\sim B_0^2a^3$ (B_0 is the surface field and a the stellar radius). For $B_0 \sim 2\times10^{12}$ gauss (ref. 13) and $a \sim 10^6$ cm, the stored energy $\sim 4\times10^{42}$ erg. Beyond this region of magnetic trapping current models, for example Rees and Gunn[14], envisage a relativistic wind flowing out radially to a shock boundary, where the outflow pressue is balanced by the gas pressure of the surrounding nebula. A release of energy from the pulsar magnetosphere, producing a temporary enhancement in the wind, would then give rise to enhanced synchrotron emission on striking the shock boundary. Although it is difficult to estimate the radius of this shock in Vela (no wisps seen, pressure in outer nebula uncertain), scaling from the Crab Nebula

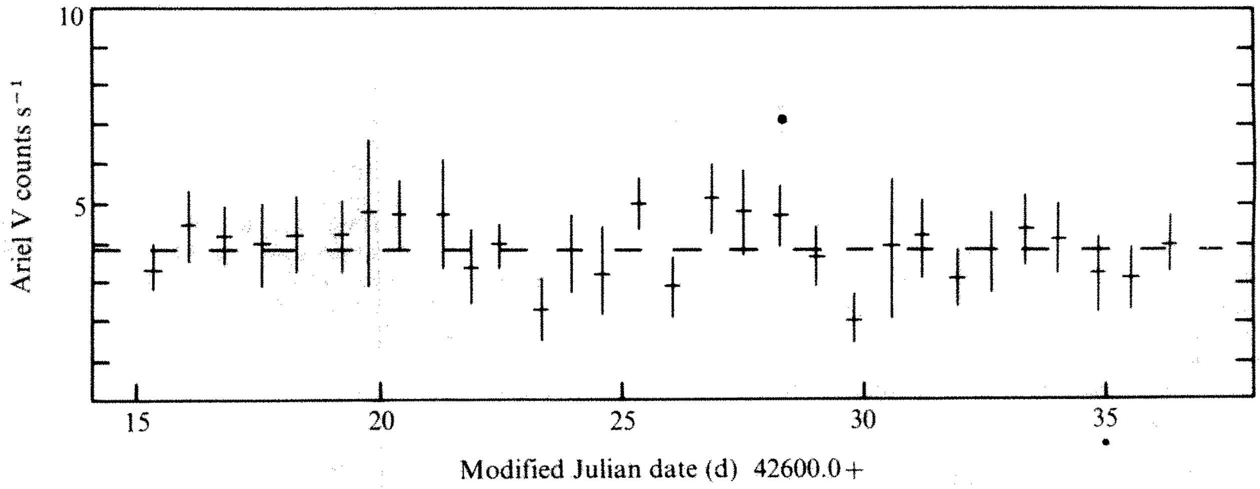

Fig. 2 0.7 d summed observations of 3U0833−45 during August 1975.

according to the ratio of mean pulsar losses (60:1), suggests $R_s \sim 5 \times 10^{16}$ cm for Vela. The observed flare timescale of ~0.5 d (10^{15} light s) may be satisfied if only part of the shock boundary is affected or, perhaps more likely, the beaming of the synchrotron X rays restricts the observed emission to that arising from the fraction of the shock surface beaming radiation in our direction.

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The missing intercloud medium and spiral galaxies

IT is the purpose of this letter first to point out that several independent lines of evidence seem to indicate that the 'intercloud medium' does not exist in its usually quoted state with density $\gtrsim 0.1$ cm^{-3} and temperature $T \sim 10^4$ K. Rather, a hot, tenuous medium ($n \lesssim 10^{-2}$ cm^{-3}, $T \sim 10^6$ K) seems more consistent with observations in the neighbourhood of the Sun. That such might be the situation has been previously suggested (see refs 1 and 2). A mechanism for producing such a medium has been proposed by Cox and Smith[3]. Second, this letter points out several implications of such a "missing intercloud medium" on the large-scale structure of spiral galaxies.

The lines of evidence pointing to the preponderance in the solar vicinity (within 1 kpc) of an intercloud medium having $n \lesssim 10^{-2}$ cm^{-3} and $T \sim 10^6$ K (which we will call "Phase III" gas) are as follows: (1) Steigman, Strittmatter, and Williams[4], studying ultraviolet interstellar absorption lines, find no correlation between distance to the illuminating star and absorption line strengths for lines which arise from 'classical Phase II' ($n \gtrsim 0.1$, $T \sim 10^4$ K) intercloud gas. Instead, Phase II absorption line strengths are correlated with spectral type, indicating that circumstellar rather than interstellar Phase II gas is being observed. Column densities and ionisation states are shown to be consistent with circumstellar gas conditions one would predict from stellar wind theory.

(2) Ubiquitous interstellar O VI absorption is observed[2]. The absorption strength is apparently correlated with distance to the illuminating star, while the radial velocities of the star and interstellar line are uncorrelated. The O VI ion is expected to be present in the ultra-hot Phase III medium, but not in the 10^4 K Phase II medium.

(3) Very stringent upper limits ($n_{\mathrm{HI}} < 0.02$) exist on the neutral hydrogen density in the directions of two hot white dwarfs detected by the Apollo–Soyuz EUV experiment at wavelengths of 50–600 Å. (refs 5, 6). Detection of a third source identified with another hot white dwarf, by another rocket-borne EUV experiment[7], has recently placed a similar upper limit on n_{HI} in a third direction. In each of the three cases, the upper limits refer to averages over distances of about 75 pc. Even more dramatic, therefore, may be the non-detection by Copernicus of interstellar Lyman α absorption in the spectra of seven B stars located in the galactic plane at distances of up to several hundred parsec[8], particularly if we follow ref. 4 and attribute many or all of the detections of interstellar Lyman α absorption in spectra of other stars observed by Copernicus to circumstellar material. It is important to note that these low neutral hydrogen densities cannot be explained as being due to highly ionised Phase II gas, since this explanation would violate the upper limit on electron densities derived from pulsar dispersion observations[9] ($n_e < 3 \times 10^{-2}$). The low upper limits for both n_{HI} and n_e are of course consistent with the Phase III picture.

(4) When an expanding supernova remnant encounters a region of very low density, the remnant may disappear as a recognisable entity. If large fractions of the interstellar medium are in Phase III, this effect should cause a deficit of large radius supernova remnants. Jones[10] has analysed the observations of remnant sizes and concludes up to 80% (by volume) of the interstellar medium is Phase III.

(5) An interstellar medium primarily composed of Phase III gas will have a galactic scale height ~1 kpc. Therefore, both the temperature and distribution of the proposed Phase III interstellar medium are consistent with the soft X-ray background[11,12].

(6) It is also interesting to note that the "observed" mean density of the gas which is purported to be the Phase II intercloud medium is inconsistent with the amount of matter traversed by, and lifetime of, galactic disk cosmic rays.[13]

(7) New 21-cm observations of the interstellar medium in the direction of bright high-galactic-latitude continuum sources (J. M. Dickey, G. E. Salpeter, and Y. Terzian, personal communication) seem to provide no evidence for the existence of a pervasive Phase II medium, although the presence of such a medium has been inferred from older observations of the same type.[14] An 'intercloud' ($T_{\mathrm{sp}} > 500$ K) contribution to the emission and absorption profiles observed definitely exists, but appears to be correlated in velocity with the cloud ($T_{\mathrm{sp}} \sim 60$ K) contribution. Much of the 'intercloud' contribution also must come from warm gas of a few hundred degrees (J. M. Dickey, E. E. Salpete and Y. Terzian, personal communication). These observations are completely inconsistent with the strict pressure-equilibrium two-phase model, and in fact seem to provide no evidence for the existence of any interstellar material not associated with clouds.

If one assumes that the mechanism which produces the ultra-hot interstellar medium in the solar neighbourhood of our Galaxy is that proposed by Cox and Smith[3] (heating by shock waves from supernovae), then we can estimate the likelihood of also finding the Phase III medium in other spiral galaxies. Cox and Smith[3] define a porosity, q, which is related to the fraction of the interstellar medium occupied by tunnels of very hot Phase III material

$$q \propto R\, n_0^{-1.7}$$

where R is the supernova rate and n_0 is the mean interstellar gas density. We take relative values of R (for various spiral types) from Tammann,[15] and assume relative values of n_0 are given by the relative values (for the various spiral types) of the ratio of H I mass to total galactic mass.[16] We find that values of q in the Sa, Sb, and Sc galaxies are all within 20% of the local value in our Milky Way, that is

$$0.8 \leq \frac{q(\text{Sa, Sb, Sc})}{q(\text{Milky Way})} \leq 1.2$$

It therefore seems that a Phase III interstellar medium (with embedded cooler clouds and circumstellar shells) could be a general phenomenon in spiral galaxies. This has significant implications for theories of star formation in spiral arms that invoke the mechanism of a galaxy-wide, density-wave driven shock wave in the Phase II interstellar medium.

Large scale galactic shocks and corresponding filamentary spiral structure can form in the interstellar gas only if the gas is driven strongly enough to force the velocity component normal to a spiral arm, $w_\perp$, to oscillate about its unperturbed value $w_{\perp 0}$ and achieve transsonic values. Studies (see refs 17 and 18) which estimate the relative speed, $w_{\perp 0}$, of the density wave pattern and the interstellar medium suggest typical values $w_\perp \leq$ 25–50 km s^{-1}. Simultaneously the effective acoustic speed, a, is $\sim$7–12 km s^{-1} if the gaseous component is largely Phase II gas, and the formation of strong shocks in this situation is rather natural.[19]

On the other hand, if the intercloud medium is predominantly 'Phase III' gas, as it seems to be in the solar neighbourhood, the effective acoustic speed, a, is $\sim$100 km s^{-1}, and the formation of strong shocks is rather unlikely except in extreme circumstances of severe forcing. This implies that either the shock wave picture of star formation in spiral arms needs to be abandoned for galaxies with predominantly Phase III intercloud medium, or that density waves in such galaxies are strongly nonlinear. (Strom *et al.*[20] have studied two galaxies which have spiral patterns in the old population stars, but lack the usual hot, young stellar population spiral arm tracers. We suggest these may be two examples of galaxies which have high enough porosity to preclude the formation of shocks and consequent star formation.)

Galaxies with Phase III gas throughout both arm and interarm regions seem likely to be quite uncommon, however. This is because the Phase III gas is formed primarily in the supernova-rich spiral arms and the cooling time for this gas is shorter than the time it takes the gas to travel between spiral arms (B. Smith, personal communication). It seems more probable, therefore, that Phase III gas dominates the intercloud medium in most spirals only near spiral arms, where most supernovae occur. The Phase III gas then cools to a Phase II medium in the interarm region. This newly cooled medium could then experience a shock on encountering the next density wave enhancement (indeed, the skewed character of spiral arm 21-cm profiles seems to imply shocks)[21]. The shock wave would produce more young stars which would result in supernovae which would in turn re-heat the interstellar medium to a Phase III temperature, thus completing the cycle. Such a situation would significantly affect the pressure and density of gas in the plane as a function of azimuthal angle, perhaps leading to an explanation of the observed lack of strong compressional effects in the spiral arm non-thermal continuum emission[22]. Of course for this type of picture to be consistent with all the observations in our Galaxy the Sun must lie near or within an active region of star formation and supernovae, where Phase III gas can be generated, such as a major spiral arm or a secondary feature or spur.

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Limit on the frequency of intergalactic supernovae

WE shall turn the simple observation that "there are no intergalactic supernovae" into a quantitative statement for the Coma cluster of galaxies. In this cluster, where at least 30% of the total luminosity lies outside the regions normally associated with individual galaxies[1–3], the absence of intergalactic supernovae can be used to derive restrictions on the nature of the luminous intergalactic material, be it a diffuse collection of stars, a background of low surface brightness dwarf galaxies, or some other form of luminous matter.

Relevant data on the supernovae within 3° of the Coma cluster centre are collected in Table 1. Most of the data came from the list of Sargent *et al.*[4], except the data on SN 1976 (*IAU Circular* no. 2921, 1976; the radial velocity from ref. 5), and the type of the parent galaxy of SN 1962i (ref. 6).

Two supernovae listed in Table 1 fall well within the Holmberg radius of the foreground galaxy NGC 4725 (SNe 1940b, and 1969h). As a luminosity class I Sb-galaxy[7] this object is expected to be a good supernova producer. In addition, it is very unlikely that the coincidence in position is a product of chance and that these supernovae belong to the Coma cluster background: within 3° from the cluster centre there are only five foreground galaxies brighter than m_{pg} = 15.0 mag (ref. 8); their combined Holmberg area covers $\approx 3\times10^{-3}$ of the total area. Even when allowance is made for fainter, unidentified foreground galaxies, the probability of a Coma cluster supernova falling by chance within the Holmberg radius of a foreground galaxy is smaller than 10^{-2}. One supernova of Table 1 (SN 1968h) belongs to an anonymous galaxy, the high redshift of which lies well outside the typical velocity interval of Coma cluster galaxies[9] and identifies this galaxy as a background-object. The supernova is most probably physically associated with this galaxy: its central distance is only 0.03 Holmberg radii. We will exclude these foreground and background objects from our discussion.

No radial velocities are available for the parent galaxies of SNe 1962i and 1963m. We adopt these galaxies as Coma cluster members because the probability of cluster membership is high for any galaxy in the field considered, and because the morphological appearance of the galaxies agrees well with the hypothesis of cluster membership. The parent galaxy of SN 1962i is quite faint: although it seems somewhat brighter than m_{pg} = 19 mag assigned by Zwicky *et al.*[6], it could be a distant background galaxy. However, the fact that the SN 1962i was brighter at discovery ($16^{m}.5$) than its parent identifies the latter as a dwarf galaxy[10], and hence as a very probable Coma cluster member. Our sample contains ten Coma cluster supernovae; of these, five have been assigned types: four as type I and one as peculiar[4].

Table 1 Supernovae within 3° of the Coma cluster centre

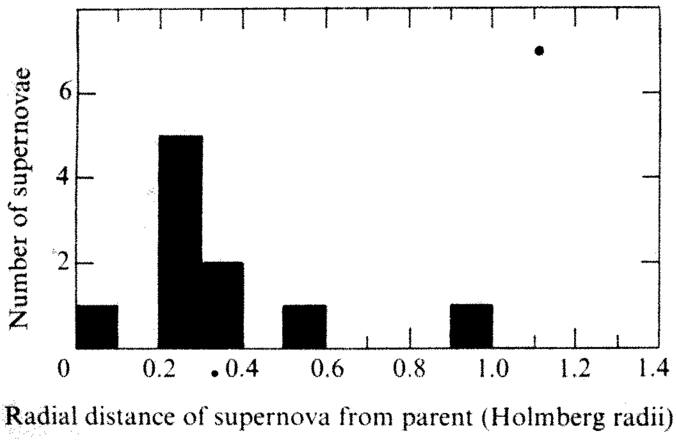
Supernova designation (1)	Suspected parent galaxy (2)	Right ascension* (3) h	min	Declination* (4) °	′	Type of parent galaxy (5)	V† (km s^{-1}) (6)	m_{pg}‡ (7)	Supernova type (8)	D_{EW}§ (arc s) (9)	D_{NS}§ (arc s) (10)	R_H∥ (11)
1940b	N4725	12	48.0	25	46	S(B)b	1114	10.2	II	95E	118N	0.22
1950a	I4051	12	58.5	28	17	E1	4932	14.8	I	2W	13N	0.23
1959b	N4921	12	59.0	28	08	Sa	5459	13.7	pec	16E	48S	0.36
1961d	Anon	12	48.3	28	06	E0	7638	14.8	I	32E	12S	0.93
1962a	Anon	13	04.3	28	08	S0	6137	16.0	I	11W	7N	0.25
1962i	Anon	13	00.5	27	47	E	—	19.0	—	8W	7N	0.56
1963c	Anon	12	55.3	28	09	E	6050	15.3	I	5W	6N	0.32
1963m	Anon	12	55.5	28	20	S0p	—	16.3	—	2W	7S	0.21
1968b	N4874	12	57.2	28	14	S0	7171	13.7	—	12W	1N	0.08
1968h	Anon	12	55.9	27	24	Sb	12050	16.6	I	9W	2N	0.03
1969h	N4725	12	48.0	25	46	S(B)b	1114	10.2	I	18E	10N	0.20
1973f	N4944	13	01.5	28	28	S0	7009	13.3	—	27E	3N	0.26
1976	N5004a	13	08.7	29	50	SBa–b	7163	15.3	—	7W	18N	0.28

*Epoch 1950.0.
†Heliocentric radial velocity.
‡Apparent magnitude of parent.
§Distances of supernova from centre of parent (east–west and north–south).
∥Radial distance of supernova from parent in units of the Holmberg radius, derived as described in text.

The radial distances given in Table 1, column 11, were calculated by assuming that the outer isophote of each galaxy is an ellipse with the major and minor axis given by the Uppsala diameters[11]. Galaxies not appearing in the Uppsala list were measured independently, and these measures were converted to the Uppsala scale. Using the Uppsala diameters and position angles and the supernova distances of Table 1, we determined the ratio of the supernova distance to the distance of the Uppsala isophote at the same position angle. These radial distances were converted to distances in units of Holmberg radii[12] using a scaling factor determined from galaxies common to both lists. Figure 1 shows a histogram of these radial distances.

To estimate the supernova rates per unit luminosity in the Coma cluster, the following quantities are needed: the luminosity in galaxies within our 3° radius, the effective number of search years, and the relative luminosity in spiral and elliptical galaxies. For the luminosity within a 3° radius, we take the value $L = 9.6\times10^{12}\,L_{\odot}$ derived from the data of Thuan and Kormendy[2] using the model proposed by King[13] to extrapolate to 3°. ($H_0 = 55$ km s^{-1} Mpc^{-1} is assumed throughout.) This value agrees within a factor of two with the values found by other observers[14,15]. Barbon[16] estimates the efficiency of the Palomar supernova search to be 90% between 1957 and 1967. Our list contains six supernovae discovered during this time, and hence one could conservatively estimate that our sample of ten Coma supernovae corresponds to 15 effective search years. This number is consistent with the fact that the Coma field was surveyed at Palomar alone during 96 different months in the years 1958–1971 (ref. 4). However, the uncertainties in this estimate are difficult to evaluate, and we consider this only as a reasonable indication of the effective search time. The ratio of luminosity in ellipticals to that in spirals can be estimated from the data for the brighter cluster members provided by Rood *et al.*[14], and we will take $L(\mathrm{E/SO}):L(\mathrm{Spirals}) = 6:1$.

Fig. 1 Histogram of radial distribution of supernova in the Coma cluster galaxies.



The above data imply the following supernovae rates for the eight supernovae in E/SO galaxies and for the two supernovae in spirals

$$\nu_c\,(\mathrm{E/SO}) = 6\pm2\ \mathrm{SNU}$$
$$\nu_c\,(\mathrm{Spirals}) = 8\pm5\ \mathrm{SNU}$$

where the units of SNU (supernova units) are SNe (100 yr)$^{-1}$ $(10^{12}\,L_{\odot})^{-1}$. These numbers are dependent on the assignment of types to the parent galaxies. If we had adopted the types from Maza and van den Bergh[7], the frequency in E/SO galaxies would have been decreased by about a factor of two and that in spirals increased by roughly the same factor. For comparison, the rates in nearby galaxies given by Tammann[17] are

$$\nu_{NB}\,(\mathrm{E/SO}) = 25\pm10\ \mathrm{SNU}$$
$$\nu_{NB}\,(\mathrm{Sa}) \approx 20\ \mathrm{SNU}$$
$$\nu_{NB}\,(\mathrm{Sc\text{-}Irr}) = 140\pm20\ \mathrm{SNU}$$

The rates for the Coma cluster galaxies may be less than for the nearby sample. This may not be statistically significant, but if it were, it might have been anticipated in view of the fact that the searches tend to discriminate against supernovae in the centres of more distant galaxies[4]. If we use the results of Sargent *et al.* on supernova frequencies as a function of the apparent magnitude of the parent galaxy as the basis for an extrapolation from Tammann's nearby galaxy sample to the distance of the Coma cluster, we would expect to find a still lower supernova rate (by a factor ~ 2) in the Coma cluster than in fact we do. Thus we conclude that the region of the Coma cluster has been quite carefully surveyed and that the galaxies are producing supernovae at about the same rate as the nearby galaxies.

An upper limit to the intergalactic supernova rate can be derived from the value for the intergalactic luminosity. We again use the data of Thuan and Kormendy[2] and the model of King[13] to derive the value $L = 5.0\times10^{12}\,L_{\odot}$ as the luminosity outside the Holmberg radii of the galaxies. This estimate agrees with that of de Vaucouleurs[1]. Since none of the ten supernovae in our sample have occurred outside the Holmberg radius of the parent, we derive a limit on the intergalactic supernova rate by assuming one intergalactic supernova

$$\nu_{IG} < 1 \text{ SNU}$$

This rate is more than six times less than our observed rate in Coma cluster E/SO galaxies and more than ten times less than the rate in nearby E/SO galaxies.

An independent limit on the relative efficiency of the intergalactic material for producing supernovae can be derived by the following argument. We observe no supernovae outside the Holmberg radius in ten examples. This would occur with 50% probability if only 6.7% of the supernova producing matter were outside the Holmberg radius. In fact, 34% of the total luminosity is exterior to the Holmberg radius and, hence, this intergalactic material is at least five times less efficient in producing supernovae than the material in galaxies.

Our entire discussion has assumed that the intergalactic region has been as carefully surveyed as the regions around the individual galaxies themselves. For the Coma cluster, we believe that this may be true since the cluster is rather compact, and the empty regions between galaxies are relatively small so that an observer would not be likely to miss supernovae even at large distances from the galaxies. As already mentioned, the searches tend to discriminate increasingly against central supernovae in more distant galaxies, and this effect should tend to increase the relative frequency of outlying supernovae. On the other hand, there is no quantitative way of estimating the search efficiencies in the intergalactic regions, so that our conclusions on the intergalactic supernovae rates could be taken, at worst, as limits on the relative survey efficiency. Clearly, we do not support this view, but cannot be quantitative and must rely on what we know and hear about how the searches are conducted. At least in the case of the Palomar search, the plates have been uniformly blinked over the entire region (C. T. Kowal, personal communication).

With the above caveat, we conclude that the intergalactic medium is deficient in supernova progenitors by a factor of $\geqslant 5$. This holds independent of the nature of the luminous material, be it the effect of overlapping haloes of E/SO galaxies, the light of undetected dwarf galaxies, or of any other origin.

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Demonstration of upper and lower Newtonian fluid behaviour in a pseudoplastic fluid

THE most common type of non-Newtonian fluid is the pseudoplastic fluid. The pseudoplastic fluid is characterised by a constant viscosity at very low shear rates, a viscosity which decreases with shear rate at intermediate shear rates, and an apparently constant viscosity at very high shear rates. That is

$$\lim_{\dot\gamma\to 0}\,[\tau/\dot\gamma] = \eta_0 \text{ (zero shear viscosity)} \qquad (1)$$

and

$$\lim\,[\tau/\dot\gamma] = \eta_\infty \text{ (infinite shear viscosity)} \qquad (2)$$

The apparent viscosity defined by

$$\eta = \tau/\dot\gamma \qquad (3)$$

then decreases with shear rate from η_0 to η_∞ and the pseudoplastic fluid exhibits a lower and upper region of Newtonian behaviour. Examples which illustrate the zero shear viscosity and the decreasing viscosity region for pseudoplastic fluids are very common and include almost all polymer melts and solutions. η_∞ is generally not well defined. Often the data available only approach the high shear rate limit and η_∞ is either determined by extrapolation or is set equal to zero which is a suggested theoretical value. Data which illustrate the three regions of pseudoplastic behaviour are rare and indeed are not quoted in the standard reference texts on rheology. The purpose of this communication is to present such data for a polymer solution and to compare the results to the models which are available for pseudoplastic fluids.

Expressions of varying complexity have been proposed to model pseudoplastic behaviour. The Meter model[1] contains η_0, η_∞ and two other parameters, τ_m and α.

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + (\tau/\tau_m)^{\alpha-1}} \qquad (4)$$

τ_m is the shear stress at which the viscosity η has dropped to $\frac{1}{2}(\eta_0 + \eta_\infty)$ and hence is another measurable quantity. α is a measure of the rate at which η is reduced from η_0 to η_∞ and can be determined from the slope of a plot of $(\eta_0 - \eta)/(\eta - \eta_\infty)$ against τ on log–log coordinates, providing both η_0 and η_∞ are known. If $\eta_\infty \ll \eta_0$ then $\tau_m = \tau_{\frac{1}{2}}$, the shear stress at which the viscosity has dropped to $\frac{1}{2}\eta_0$, and the Meter model reduces to the Ellis model[2].

$$\eta = \frac{\eta_0}{1 + (\tau/\tau_{\frac{1}{2}})^{\alpha-1}} \qquad (5)$$

which in turn shows power-law behaviour at high shear stresses

$$\eta = K\tau^{1-\alpha} = \eta_0(\tau_{\frac{1}{2}})^{\alpha-1}\tau^{1-\alpha} \qquad (6)$$

Three instruments, a Brookfield viscometer, a Weissenberg rheogoniometer, and a gas-driven capillary rheometer were used to make shear stress–shear rate measurements over a large enough shear rate range to illustrate upper and lower Newtonian behaviour. A 0.4% weight polyacrylamide solution was used. The Brookfield viscometer with a cylindrical spindle of length 6.36×10^{-2} m and diameter of 1.87×10^{-2} m was used to measure the shear stress as a function of shear rate at low shear rates. Relative to the diameter of the spindle, the fluid medium in which the spindle was immersed was infinite in size. End

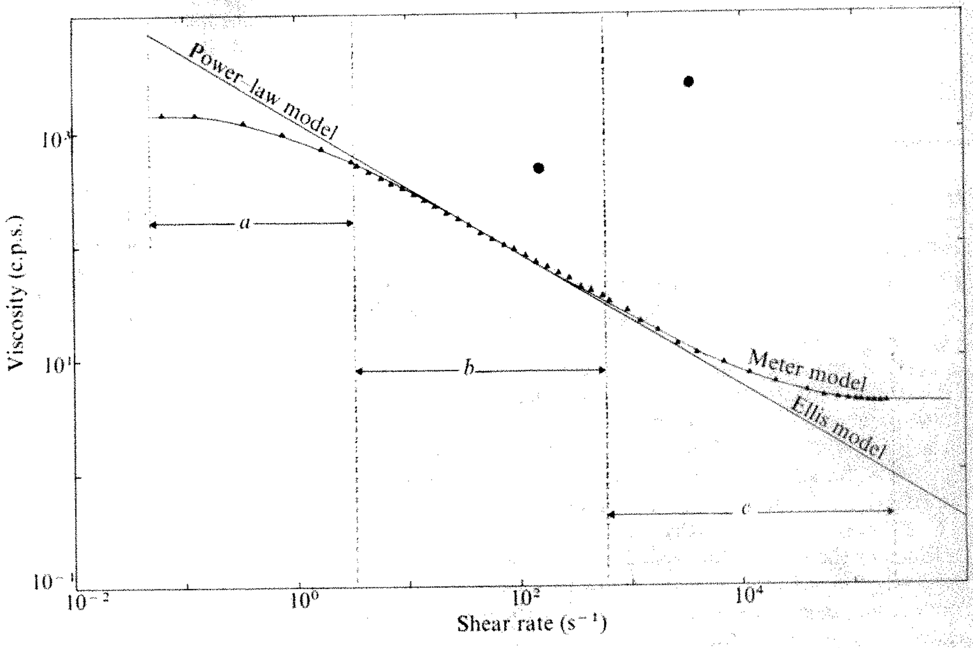

Fig. 1 Illustration of upper and lower Newtonian behaviour for a 0.4% polyacrylamide solution. *a*, Brookfield viscometer; *b*, cone and plate viscometer; *c*, capillary viscometer.

corrections were less than 10%. For this condition the shear stress and the shear rate on the surface of the spindle are given by ref. 5.

$$\tau_b = \frac{T}{2\pi R_b{}^2 L} \tag{7}$$

$$\dot{\gamma}_b = \frac{4\pi N}{n''} \tag{8}$$

where T = measured torque; R_b = radius of spindle; L = length of spindle; N = rate of revolution of the spindle (r.p.s.); n'' = slope of a log–log plot of τ against N for each value of τ.

A 2°1′26″ cone was used with a 0.075 m diameter plate in the Weissenberg rheogoniometer. For the small cone angle employed the shear stress and hence the shear rate do not vary in the gap between the cone and plate and the shear stress and shear rate are related to the torque exerted on the plate and the rate of rotation of the cone by[6]

$$\tau = \frac{3T}{2\pi R^3} \tag{9}$$

and

$$\dot{\gamma} = \Omega/\alpha \tag{10}$$

where R = radius of the platens; Ω = angular velocity of the cone; α = cone angle.

A gas-driven capillary rheometer was used for the high shear rate region. The tube internal diameter was 5.29×10^{-4} m and its length was 0.3789 m. Exit and entry pressure losses are negligible for such a high L/D capillary tube and the wall shear stress and wall shear rate are defined in terms of the measured pressure drop (Δp) and volumetric flow rate by[5,6]

$$\tau_w = \frac{R\Delta p}{2L} \tag{11}$$

$$\dot{\gamma}_w = \frac{3n+1}{4n}\left(\frac{4Q}{\pi R^3}\right) \tag{12}$$

where n is the slope of the log–log plot of τ_w against $4Q/\pi R^3$ at each value of $4Q/\pi R^3$.

Equations (7), (8), (9) and (10) were then used to reduce the torque as a function of angular velocity data, while equations (11) and (12) were used to express the pressure drop flow rate data obtained with the capillary rheometer in terms of the wall shear stress and wall shear rate. The results obtained with the three instruments are shown in Fig. 1 where the apparent viscosity defined by equation (3) is plotted against the shear rate. The polyacrylamide solution showed no appreciable shear degradation at the high shear rates employed in the capillary rheometer.

The results in Fig. 1 cover almost six decades of shear rate and the reproducibility between the various instruments is excellent. Upper and lower Newtonian behaviour is clearly illustrated with η_0 = 1425 cP and η_∞ = 4.0 cP. Also shown on Fig. 1 are the Meter, Ellis, and power-law model fits of the experimental data with τ_m = 1.215 N m^{-2}, α = 2.43 and $\tau_{\frac{1}{2}}$ = 1.211 N m^{-2}. Notice that the Meter model is an excellent representation of the data over the entire shear rate range, the Ellis model is inadequate for shear rates in excess of 250 s^{-1} and that the power-law model is strictly valid only for 25 s^{-1} $\leqslant \dot{\gamma} \leqslant$ 250 s^{-1}. The results also clearly illustrate the importance of measuring the flow properties of a pseudoplastic fluid in the range of shear rates for which the data is to be employed and further illustrate the importance of choosing the appropriate instrument and flow geometry for making these measurements. Clearly the measurement of the flow properties of a pseudoplastic fluid or for that matter any non-Newtonian fluid in a shear rate range other than that where the data is employed is a futile exercise.

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Exact modelling of cubic lattice permittivity and conductivity

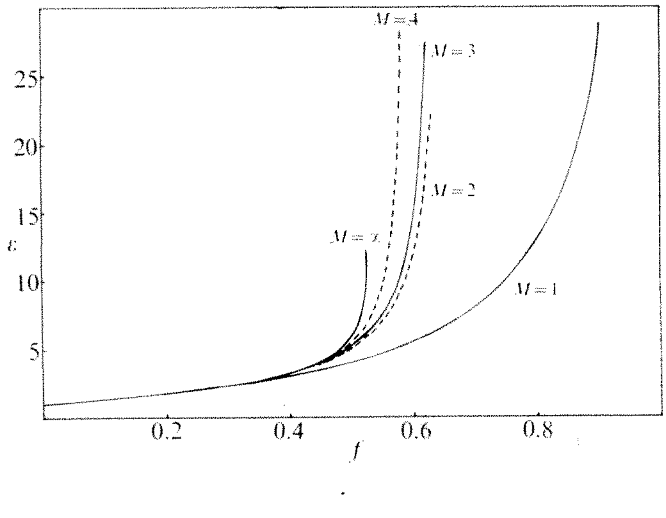
A PROBLEM of practical importance in a wide variety of situations is that of calculating the permittivity (or equivalently, the conductivity) of simple cubic arrays of spheres embedded in another material. Applications occur in, for example, the analysis of optical properties of inhomogeneous films[1], dielectric dispersion in mixtures[2], the behaviour of artificial dielectrics[3], the study of lunar soil samples[4] and in vision research[5]. Although the problem is an old one, and has attracted the attention of some of the great names of physics (for example, J. C. Maxwell[6] and Lord Rayleigh[7]) it seems that a completely satisfactory theory has not existed until the present time. This is shown by the fact that in the most critical case, that of perfectly conducting spheres, none of the existing theories give the expected divergence at the critical volume fraction ($f_c = \pi/6$) at which the spheres touch. We have extended Lord Rayleigh's treatment and present a theory which gives the correct behaviour for all volume fractions.

The first order (dipole) solution to the problem is known as the Lorentz–Lorenz equation or the Maxwell Garnett equation

$$\varepsilon = 1 - \frac{3f}{[(2+\varepsilon_1)/(1-\varepsilon_1)]+f} \tag{1}$$

where ε, ε_1 are, respectively, the permittivities of the resultant medium and of the spherical particles (both measured relative to the permittivity of the matrix); f is the volume fraction of the particles. The range of f over which equation (1) is accurate is not clear. This is a question of some importance in that controversy exists as to the cause of discrepancies between equation (1) and optical measurements made on samples with $f > 0.3$. Some authors attribute the discrepancies to variation of ε_1 with particle radius[8] while others invoke various distributions of particle shape and orientation[9]. We consider that, before these two hypotheses can be meaningfully tested, proximity (higher order multipole) effects should be taken into account.

Fig. 1 Permittivity ε of a simple cubic array of perfectly conducting spheres as a function of volume fraction f. The order of the theory is indicated. $M = 1,2$, and 3 correspond respectively to the theories of Maxwell Garnett, Rayleigh (corrected) and Meredith and Tobias. $M = 4$ corresponds to equation (3) and $M = \infty$ to the exact curve.



In his classic paper[7] Rayleigh gave a second order treatment, with the aim of determining the region of validity of (1) for the cubic lattice. He took into account the effect of octupoles as well as dipoles (only 2^{2M-1} poles with M integral being allowed by symmetry). It is unfortunate that Rayleigh made two simple errors in the derivation of his final formula. The first of these (the omission of a factor of π) was corrected by Runge[10]; the second (the omission of a factor of 2/5 which arises in a partial differentiation of his equation (61)) is corrected here.

Lord Rayleigh's treatment was extended by Meredith and Tobias[11] to include the effects of poles of order 2^5. We have derived a formula which includes the effects of poles of order 2^7:

$$\varepsilon = 1 - 3f/D \tag{2}$$

$$\text{where } D = T_1^{-1} + f - b_1 T_5 f^{14/3} - c_1 T_7 f^6$$
$$- af^{10/3}\,\frac{[1 - c_2 T_5 f^{11/3} + c_3 T_5^2 f^{22/3}]}{[T_3^{-1} + b_2 f^{7/3} - c_4 T_5 f^6]} \tag{3}$$

Here

$$T_n^{-1} = \frac{\varepsilon_1 + [(n+1)/n]}{1-\varepsilon_1} \tag{4}$$

and

$$a = 1.3045 \tag{5}$$

$$\left.\begin{aligned} b_1 &= 0.01479 \\ b_2 &= 0.4054 \end{aligned}\right\} \tag{6}$$

$$\left.\begin{aligned} c_1 &= 0.1259 \\ c_2 &= 0.5289 \\ c_3 &= 0.06993 \\ c_4 &= 6.1673 \end{aligned}\right\} \tag{7}$$

Full details of the derivation of these constants (which included the re-evaluation of certain sums) will be presented later. Equation (3) yields the formula of Meredith and Tobias if c_1 to c_4 are replaced by zero. The corrected equation of Lord Rayleigh results if in addition b_1 and b_2 are replaced by zero.

Table 1 f_c and $f_{\max}$ as a function of M, the number of terms in the multipole expansion

M	f_c	$f_{\max}$
1	1.000	0.30
2	0.66505	0.41
3	0.64469	0.43
4	0.59250	0.46
10	0.54260	0.48
25	0.5279	0.515
50	0.5248	0.522
100	0.5239	

We have generalised the formalism in such a way as to permit its programming to any desired order of the multipole field expansion. Figure 1 shows the comparison of results provided by our programme for theories of increasing order M. Note that only the highest order theory has its divergence at the correct value of f. This point is illustrated more explicitly in Table 1, which also shows the upper limit on f, $f_{\max}$, at which the theory of order M is correct to within an absolute tolerance of 5×10^{-2}.

While a great deal of experimental information exists on disordered arrangements of spheres, we know of only two sets of measurements on simple cubic arrays of perfectly conducting particles[3,11]. These are compared with the results of theory in Fig. 2. The theoretical curve has been tested for convergence and is accurate to better than 1%. The complete agreement between theory and experiment is evident. It is interesting to note that the experimental measurement made closest to the touching point, with $f = 0.5161$, differs from theory by less than 0.1. We have shown numerically that to obtain this agreement we must include multipoles of order up to 2^{29}. This is a tribute to the remarkable accuracy of the measurement.

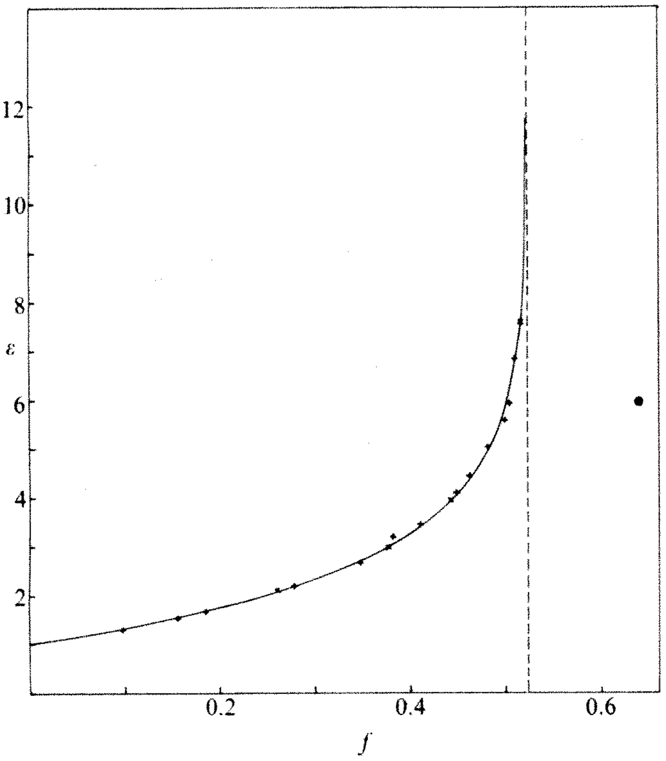


Fig. 2 Experimental measurements of Kharadly and Jackson (+) and of Meredith and Tobias (×) compared with the exact theoretical curve ($M = \infty$) of Fig. 1. The vertical dashed line corresponds to $f = 0.5236$.

Although we have discussed here only results pertaining to real permittivities of the spheres, the theory is general and its application to complex permittivities will be discussed later. We also envisage its extension to the close-packed lattices with the aim of devising a formula for close-packed random arrays. It is evident from the results above that such a formula is necessary to supplant the Maxwell Garnett equation (1) for dealing with random arrays having a volume fraction f greater than about 0.3.

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Surface circulation in Liverpool Bay

CONCERN over the influence of industrial and domestic effluents on the coastal waters of Liverpool Bay has led over the last decade to a number of physical, chemical and biological surveys[1–7]. Despite this intensive effort there is little that can at present be stated with confidence about the form and variability of the circulation (A. J. Bowen, T. R. S. Wilson and M. J. Howarth in ref. 1, vol. 3, pages 45–55). Conclusions derived from direct observations of residual currents (refs 2 and 3 and J. W. Ramster in ref. 1, vol. 2, pages 56–64) and indirect inferences from plankton[4] and chemical distributions[5–7] have combined to produce strong evidence in favour of either a clockwise[2,4] or anticlockwise (refs 3, 5–7 and J. W. Ramster in ref. 1, vol. 2, pages 56–64) surface residual circulation.

Direct observations of current direction and speed can be obtained over protracted periods from either continuously recording current meters (A. J. Bowen, T. R. S. Wilson and M. J. Howarth in ref. 1, vol. 3, pages 45–55; and J. W. Ramster in ref. 1, vol. 2, pages 56–64) attached to fixed buoys or from radio-tracked parachute drogues[8]. The obvious difficulty here is the sparse spatial distribution of synoptic data that can be obtained on anything other than a local scale. An alternative approach to elucidate the direction if not the magnitude of the circulation is to make use of the distribution of salinity, temperature and conservative chemical characteristics of the waters. The potential of utilising the intrinsic and quite often unique chemistries of the source waters of Liverpool Bay in any quantitative sense has largely been ignored[7]. This presumably is a consequence of the relatively large number of source waters that may significantly contribute to the area, and hence the number of different tracers required for the solution of any mixing model, together with the large number of samples that would have to be collected in this area of highly heterogeneous water chemistry[9] to provide comprehensive and unambiguous distributions.

Our objective has been to use the distributions of physical and chemical characteristics to determine the surface residual circulation pattern in Liverpool Bay. Dissolved concentrations of silicate, phosphate, nitrate, nitrite and ammonia were chosen as potential tracers in conjunction with salinity and temperature. The chemical species clearly do not meet the conservative prerequisite of a natural tracer during periods of sustained primary production and hence can only be used during the winter period. They can, however, be analysed by continuous on-line techniques[9,10] which provide greater definition of distributions and allow unambiguous interpretations that are not often possible with more traditional methods based on sampling at discrete stations.

Continuous records of the surface variability of these seven characteristics were obtained during a number of cruises on a comprehensive grid through Liverpool Bay during the winters of 1974 and 1975. Detailed descriptions of the observed distributions will be reported elsewhere. The number of contributory source waters and end member properties on each cruise were obtained by a combination of principal component analysis[9-11] and examination of the envelopes enclosing 21 X–Y diagrams generated by sequential plots of coincident data from the continuous records. On all cruises so far conducted the number of major sources identified has been smaller than the number of characteristics monitored, permitting a number of mixing model solutions from the same data set simply by varying the combination of tracers used.

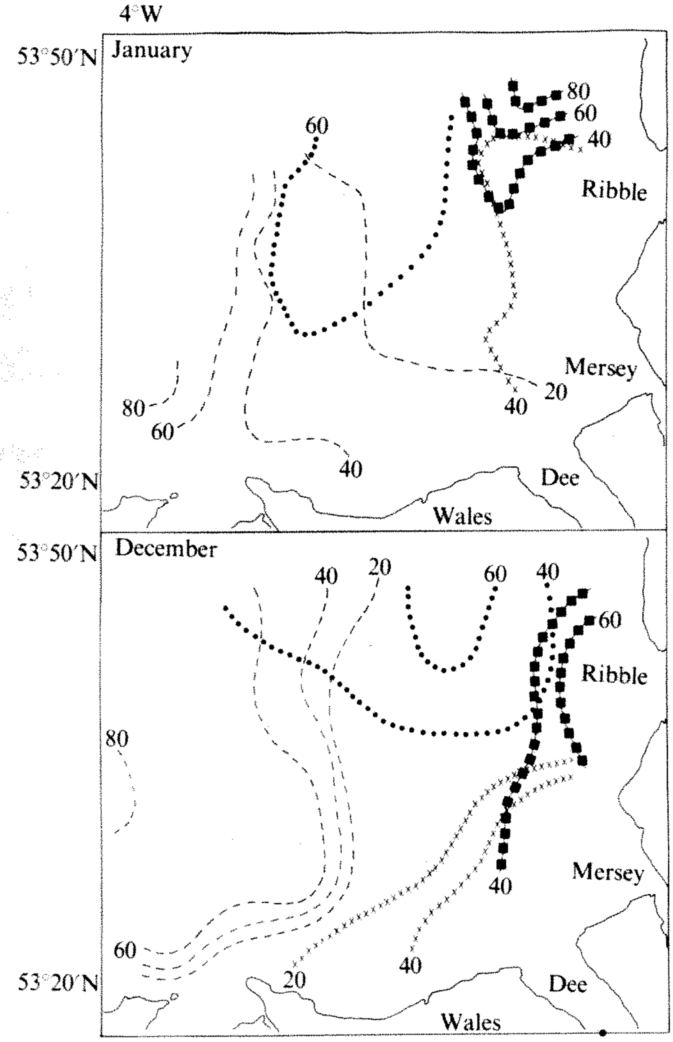

Fig. 1 During both January and December four major water types were identified. Mixing model solutions, showing percentile distributions are illustrated, both derived from salinity, temperature and silicate data. – – – Western boundary water; ●, northern boundary water; × and ■, fresh water.

Model solutions for the cruises so far conducted have produced two distinct extremes, observed during January and December 1975. The solutions are illustrated in Fig. 1, which shows that (1) in January, western boundary water encroached well into Liverpool Bay along the North Wales coast, in contrast to December when a marked physical discontinuity was observed at approximately 3°50′W. (2) In December a significant contribution from fresh water sources persisted along the North Wales coast, whereas in January a northerly transport of these waters was apparent, (3) in both January and December, a significant contribution to the waters in the centre of the bay originated from the northern boundary. Figure 2 shows the suggested circulation patterns. This novel approach to elucidation of the circulation pattern of Liverpool Bay demonstrates that either an anticlockwise or a clockwise circulation of the surface waters may be experienced during the winter period.

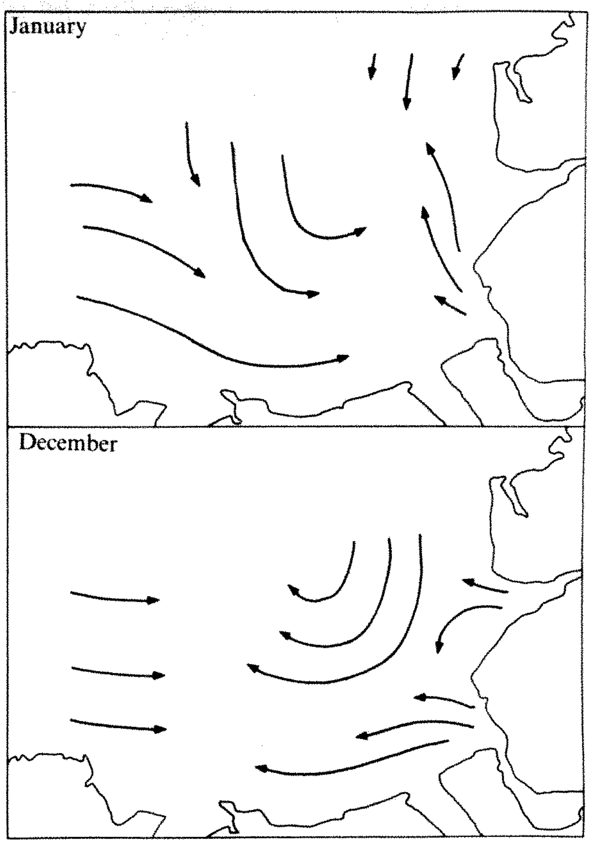

Fig. 2 Suggested surface circulation patterns in Liverpool Bay, January and December, 1975.

Of particular significance is the sharp contrast in the spatial distributions of potential pollutants associated with each circulation mode. Surface distribution maps exhibited high pollutant concentrations over an extensive sea area during periods of sustained clockwise circulation. We conclude that the replacement time of the fresh waters (particularly from the River Mersey with its associated industrial and domestic effluents) discharged into Liverpool Bay during periods of clockwise surface circulation is significantly greater than when the motion is anticlockwise. The possibility of deleterious consequences in the marine regime resulting from the terrestrial discharges is intimately related to the factors which initiate and subsequently maintain the clockwise circulation mode. Elucidation of these factors is the objective of continuing cruises.

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Cyclonic ring formation at the polar front in the Drake Passage

THE first summary of geographical locations of the antarctic convergence (polar front) by Mackintosh[1] showed that >50% of the instantaneous locations of the front differed from the mean location by as much as ±50 km. Early hydrographic section work could not resolve the temporal or spatial fluctuations in the horizontal structure of the front. During FDRAKE 75 Gordon[2] observed comparable fluctuations in the frontal position in the Scotia Sea but again the spacing between sections was too wide. During FDRAKE 76 (refs 3, 4 and T. M. Joyce *et al.*, unpublished) the small scale structure of the polar front was studied in the Drake Passage. Two ships, the RV Thompson (University of Washington) and the Chilean naval vessel AGS Yelcho, combined efforts in the program which took place during the onset of the austral autumn. We describe here the evolution of the polar front between February 29 and April 5, 1976, a time span of ~5 weeks.

The polar front, in reality a transition zone between antarctic and subantarctic water masses[5], is most clearly revealed in vertical sections of temperature as that from our data shown in Fig. 1. South of the frontal zone there is a temperature minimum layer between 100 and 300 m. As noted by Gordon[6], this feature is insulated from seasonal heating at the surface. Within the frontal zone the minimum deepens abruptly and erodes splitting into multiple extrema of interleaving antarctic/subantarctic waters. Although numerous definitions of the polar front have been suggested[7], the operational definition used in our analyses is the presence of a well defined temperature minimum layer as delineated by the 2 °C isotherm. The selection of the 2 °C isotherm is somewhat arbitrary, but it does provide a convenient, consistent, and objective method for defining the position of the southern edge of the frontal zone.

Synoptic maps of the front have been produced with the combined data from the two vessels. While some artistry is involved in smoothing the observations, the horizontal density of measurements is sufficient to resolve the structure. A composite of these maps, Fig. 2, indicates that between March 10 and 24 a large meander developed in the polar front. This is verified by thermistor chain measurements (position shown in Fig. 2) from a subsurface mooring[8] in the vicinity and by the distribution of surface properties (not shown). The meander subsequently pinched off between March 28 and 30 into a cold, cyclonic ring of ~60–80 km diameter which then drifted to the NE at a speed of ~10 cm s^{-1}. The ring formation process is reminiscent of that observed in western boundary currents such as the Gulf Stream[9]. Continuous profiles of temperature and salinity show that the antarctic water types inside the ring penetrate to a depth of 2,500 m (Fig. 3), suggesting that much of the water column is involved.

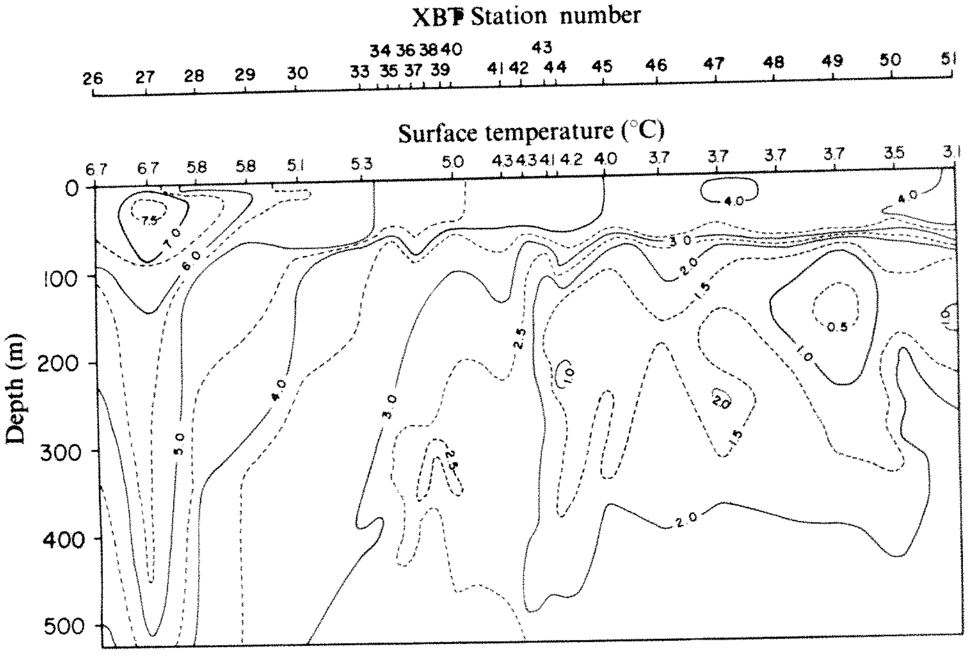

Fig. 1 XBT section across the polar front. Contours are in °C. Starting and ending positions for section are: XBT 26 (56° 44.8′ S, 65°30.4′W), XBT 51 (59°1.95′ S, 63° 2.5′ W).

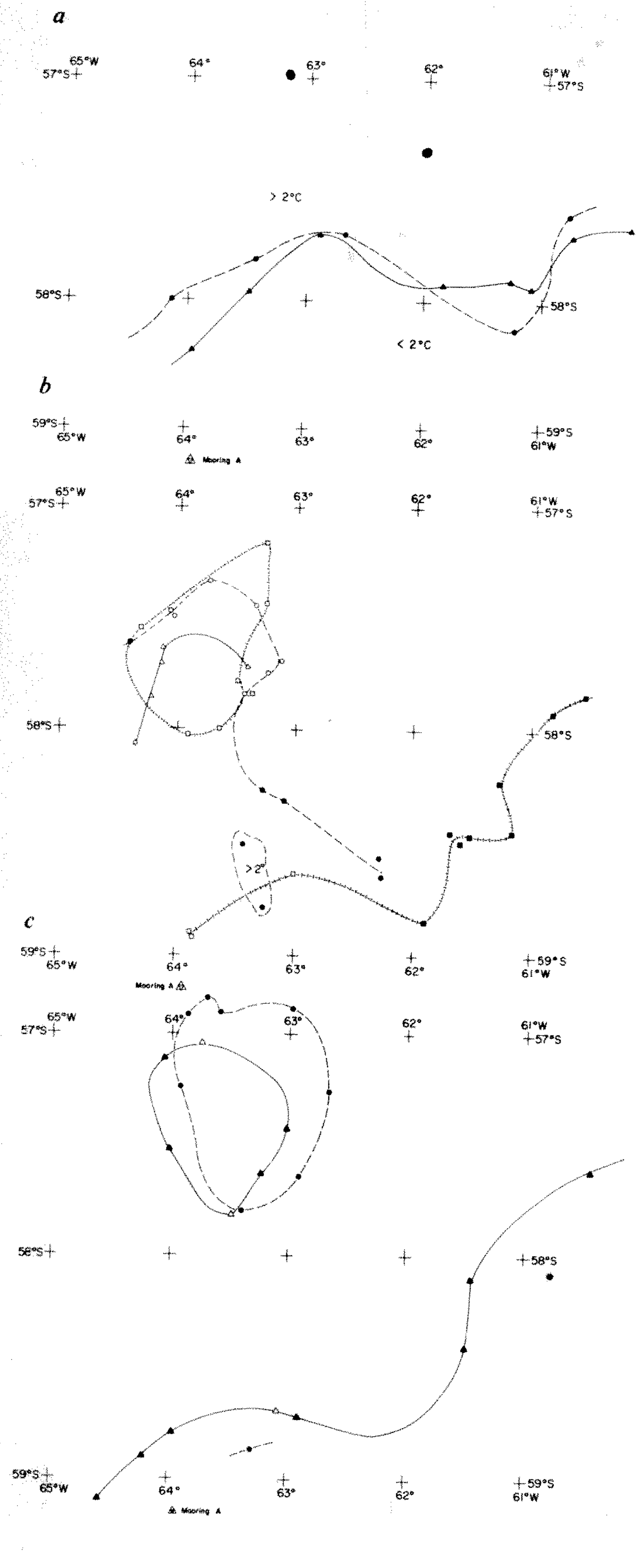

Fig. 2 Evolution of the polar front. Shown contoured is the boundary between antarctic waters and the interleaving regime of the frontal zone. Times are coordinated universal (CUT). *a*, February 29, 0130–March 5, 1830 △, ———; March 8, 1330–March 9, 1540 ○, — — —. *b*, March 16, 0000–March 20, 2359 △, ———; March 21, 0000–March 26, 2359 □, — — —; March 27, 0000–March 30, 2359 ○, + + + + .*c*, March 31, 0000–April 3, 2359 △, ———; April 4, 0230–April 5, 1315 ○, — — —. Solid (open) symbols represent observations from the Yelcho (Thompson).

Subsurface neutrally buoyant floats equipped with fins to react to vertical water motion[10,3], were tracked acoustically from the RV Thompson during the period of the ring formation. The track of these floats (Fig. 4) indicates that three of the four vertical current meters (VCM) were entrapped in the ring while the fourth continued unaffected to the NE. Horizontal velocities of the floats were 30–40 cm s^{-1}. The depth of each VCM at launch and recovery is indicated in Fig. 4.

A series of CTD (conductivity, temperature, depth) stations to 3,000 was made directly through the ring by the RV Thompson. These data, together with nutrients and dissolved oxygen need to be analysed before a complete description of the eddy properties (for example, circulation) can be determined. This work is in progress.

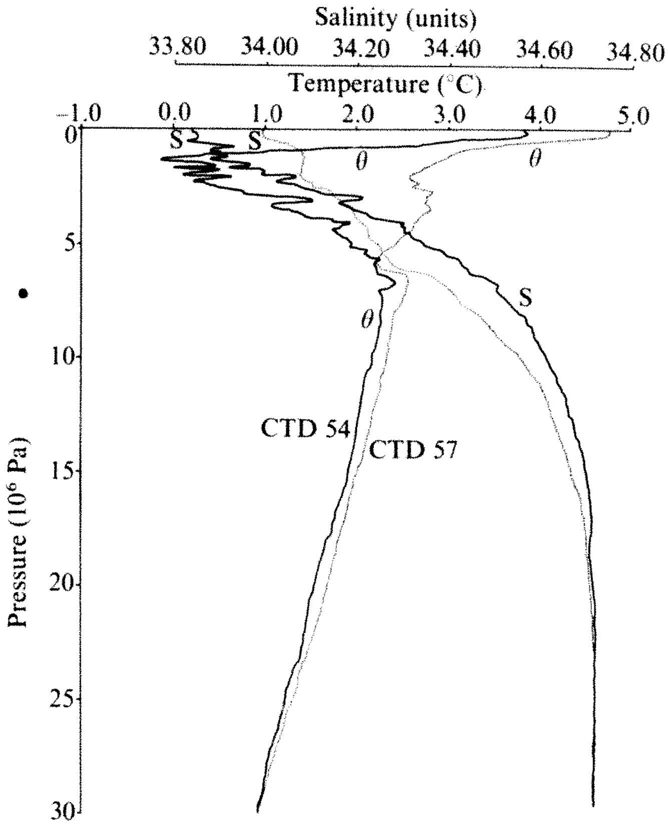

Fig. 3 Conductivity, Temperature, Depth (CTD) stations inside (54) and south, outside of the ring (57) but still north of the re-established polar front. Station locations are: 54: 57°39.9′ S, 63°51.0′ W, and 57: 57°59.9′ S, 62°59.8′ W.

The process described here can potentially transport large amounts of antarctic or subantarctic water across the front and may be fundamental to the understanding of heat and salt transfers towards the polar region. If the cold ring is reabsorbed into circumpolar current, the net transfer of properties will be less than if the ring were to remain detached, slowly decaying with time. Downstream of the Drake Passage, the circumpolar current system is highly constrained by the Scotia Ridge[11] which has only two large openings with a sill depth >2.5 km. It is interesting to speculate upon the fate of our cold water ring when it drifts into this rather formidable topography.

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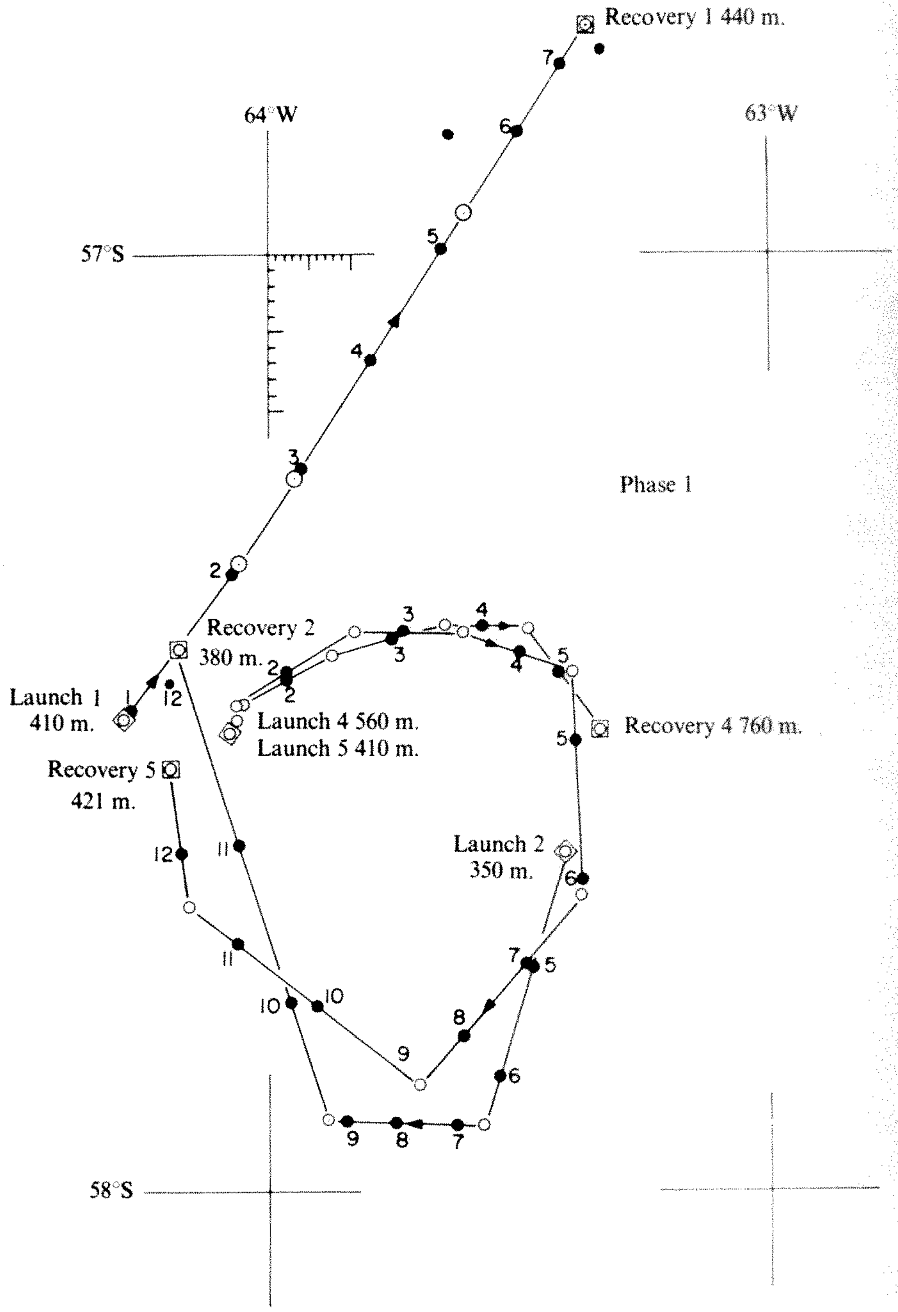

Fig. 4 Vertical current meter trajectories during cruise. Phase I was during the cold ring formation. The meander pinched off before March 30, when the float tracks began to close. ◇, launch; ⊡, recovery; ○, visit; ● 12 h time marks starting 0000 March 24.

Chilean Navy) on board the RV Yelcho. This work is part of an international southern ocean studies (ISOS) program of the NSF.

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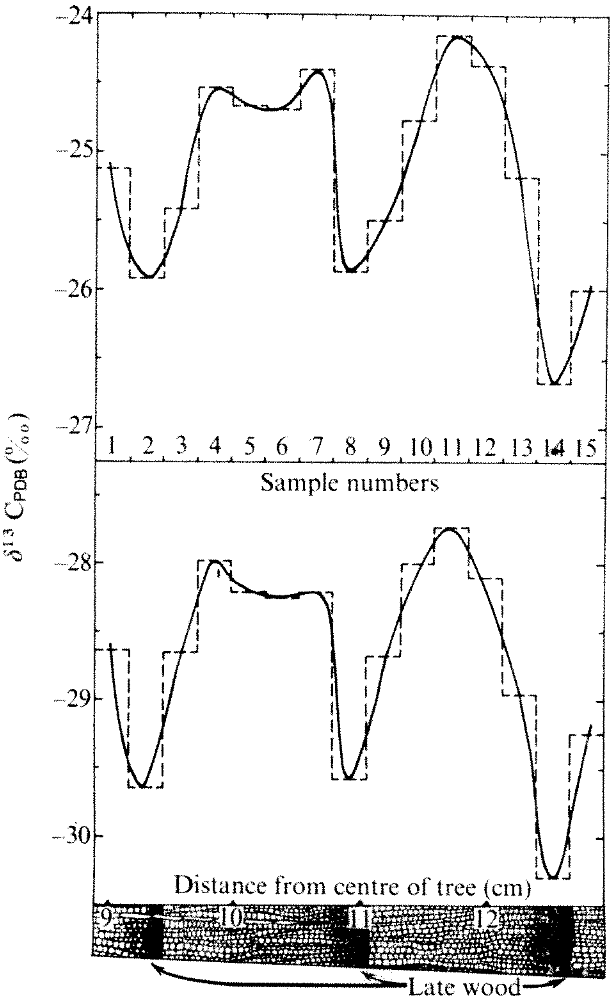
$^{12}C/^{13}C$ in cellulose and lignin as palaeothermometers

SEVERAL workers[1-3] have attempted to retrieve palaeoclimatic information from the $^{12}C/^{13}C$ ratios in tree rings. Their use of whole wood, however, has been unsatisfactory, in that the extractives are isotopically much lighter than cellulose[4] and vary considerably in content between heartwood and sapwood. Further, lignin is usually ~ 3‰ lighter than cellulose and it is well known from the extensive literature on the chemistry of wood that the lignin-to-cellulose ratio varies not only across one ring, that is, in late wood as compared to early wood, but up and down and radially across the tree[5]. The science of dendroclimatology is based on the observation that narrow rings represent a cold (or dry) year. In most conifers the amount of late wood remains relatively constant and it is the thickness of early wood that varies. A narrow ring would be expected to have a smaller proportion of early wood than a wide ring. The different proportions of lignin in the two rings would then lead to a different isotopic ratio for the whole wood. If whole wood is used the $\delta^{13}C$ measurements will reflect the relative proportions of lignin and cellulose. We therefore separated the lignin and cellulose from the wood before making $\delta^{13}C$ determinations, hoping that the temperature coefficients of their production reactions might be large enough to enable the temperatures at which the carbon was fixed from the atmosphere to be measured. We also wished to investigate the possibility that the difference in $\delta^{13}C$ between the cellulose and lignin might reflect temperature.

Preliminary experiments indicated that any temperature effects would be small; probably towards the lower limit of current mass spectrometric techniques. We therefore studied the annual rings of a specimen of *Pinus radiata* (Monterey Pine) which had grown in Hamilton, New Zealand. This tree has the advantage of laying wood all year[6], that is, over a temperature range of 10 °C. This enabled wood to be sampled that grew over a much wider temperature range than the range of post-glacial temperature fluctuations (perhaps 1–2 °C).

Fifteen samples over three annual rings were separated into cellulose and lignin using the conventional techniques of wood chemistry[7]. The samples were burned to produce CO_2 and this was measured in a Micromass 602C. The reproducibility of our measurements based on 12 separate combustions of the sample was ±0.04‰ (1σ). From Fig. 1 it can be seen that there is a depletion in ^{13}C for late (winter) wood as compared with early (summer) wood for both cellulose and lignin. Keeling[8] has measured the isotopic ratio of atmospheric carbon dioxide over the Pacific Ocean and shows that there is an annual fluctuation of ~ 0.3‰, the atmosphere becoming isotopically heavier in the late summer and autumn. This effect was attributed by Keeling to the activity of land plants[8]. The annual change we measured is of different phase and an order of magnitude greater than the change in the atmosphere. Thus we consider that the variations of $\delta^{13}C$ shown in Fig. 1 are caused by changes in air temperature affecting one or more of the biochemical reactions leading to cellulose and lignin. During the year in Hamilton both the monthly mean maximum daily temperature and the monthly mean daily temperature vary through a cycle with an amplitude of 10 °C. Thus we obtain a temperature coefficient for both cellulose and lignin of at least 0.2‰ per °C.

Fig. 1 Variation in the isotopic ratio of the carbon in cellulose (*a*) and lignin (*b*) across tree rings from *Pinus radiata*.



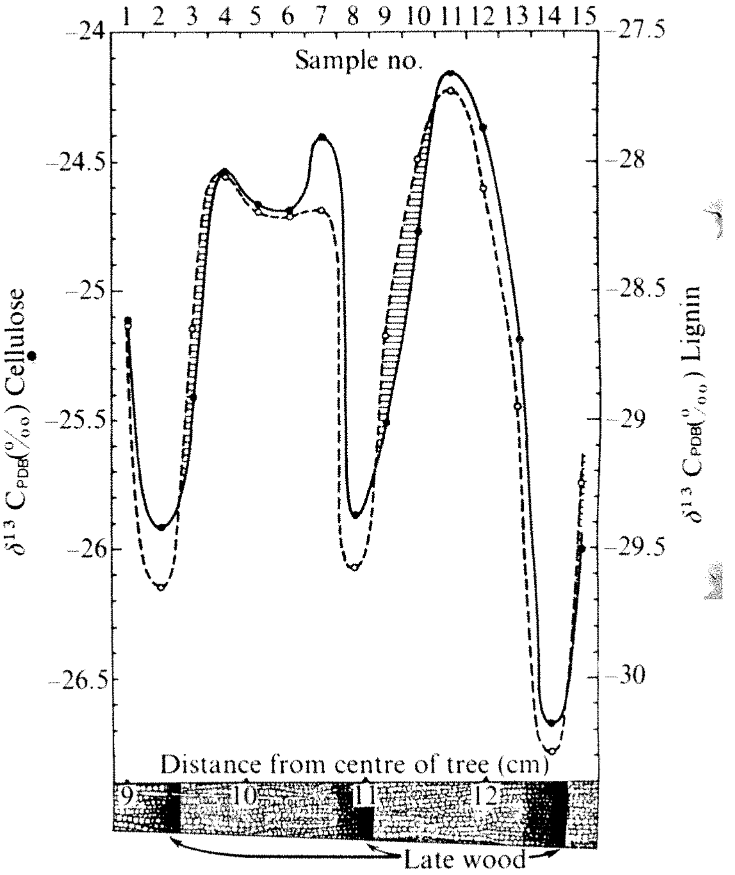


Fig. 2 Variations in the isotopic ratio of carbon across tree rings from *Pinus radiata*—a comparison between cellulose (—) and lignin (- - -).

Figure 2 presents the cellulose and lignin data superimposed. Several conclusions are evident. First, the amplitude of the $\delta^{13}C$ values of cellulose and lignin are very similar. This probably can be taken as evidence that the temperature dependent steps in the biochemical pathway leading to both cellulose and lignin are early in the sequence, probably in the leaves. Thus the temperature being monitored is that of the leaves rather than the trunk of the tree.

Second, although the cellulose and lignin curves are significantly different, these differences are probably too small to be of any use in palaeoclimatology with present mass spectrometric techniques. The third interesting result is the phase relationship between the cellulose and lignin curves. It is generally believed by plant physiologists that xylem cells are laid down and some time later lignified[9]. In Fig. 2 we can see the result of this secondary lignification, in that the lignin is significantly displaced in time and hence temperature with respect to the cellulose. This is excellent confirmation of the general interpretation given above.

If, as our results seem to indicate, the $\delta^{13}C$ values of cellulose and lignin vary with temperature, we may now ask if they will be of any use in determining past climate. Modern mass spectrometric techniques rarely measure $\delta^{13}C$ to better than 0.1‰ and few people have claimed to have made measurements with a standard deviation of better than 0.06‰ (0.3 °C). Modern mass spectrometers (such as Micromass 602C) can measure a sample of pure CO$_2$ with an s.d. of 0.02‰. The problem is associated with combustion of the sample, and in particular, impurities that contribute to the mass 45 peak in the mass spectrometer and hence limit the accuracy. We have found that with very careful combustion, great care in removing any water (which can form COOH$^+$ of mass 45) and by repeated sampling, one can make measurements with a precision of ± 0.02‰ (1σ), that is, measure temperatures to 0.1 °C. This would be adequate for palaeoclimatic studies provided one could assume that the δ^{13}C of the atmosphere has remained constant or is known.

This problem has been studied by Keeling[8,10] and others. Clearly there is an effect arising from man's combustion of fossil fuels since the Industrial Revolution. There are also localised effects in the vicinity of large industrial centres, and seasonal effects which have been attributed to the activity of land plants[8,10]. This latter effect would be averaged out if one sampled wood from a number of years. Another problem that must be avoided is that the atmosphere near the ground in a forest can be depleted in ^{13}C. This has been attributed to root and mycorrhizal respiration utilising photosynthates which are depleted with respect to the atmosphere. It has been observed that young plants growing in such a situation and therefore the internal rings of large trees are depleted in δ^{13}C (refs 1, 11, 12). Once the tree reaches maturity—perhaps because its foliage reaches the free atmosphere—the δ^{13}C content stabilises. Data from the inside rings of large trees will have to be treated with suspicion or the 'juvenile' effect—which is probably a smooth curve—corrected for. But, provided trees are chosen from suitable sites and corrections are applied for any man-made effect, it seems that δ^{13}C measurements on cellulose and/or lignin hold promise for obtaining past temperatures from subfossil wood. Since in some areas tree-ring records extend back annually for up to eight millennia, the prospect is exciting.

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Precambrian evolution in a stratified global sea

HARGRAVES[1] postulates that continents on Earth emerged from a circumglobal ocean perhaps as recently as $1.0–1.4 \times 10^9$ yr ago. Without arguing the merits of the geological evidence supporting this theory, we suggest that the heuristic value and biological implications of this hypothesis are remarkable.

If such a sea existed some 3×10^9 yr ago, then life must have originated in this environment. In conformance, then, with current theories concerning the conditions necessary for life's genesis[2] we would expect high concentrations of both reduced organic carbon compounds and nutrients such as phosphate. Although physical conditions in such an ocean during the formative stages are arguable, surface heating by solar radiation and wind mixing would, in all probability, produce a warm low density surface layer. Without continents to obstruct horizontal oceanic currents or differentially heat the Earth's atmosphere, heat distribution on the planet's surface would be more uniform than at present. The high latitude generation of deep water would be less pronounced. While equatorial upwelling might still be expected, its magnitude is not clear and certainly the prominent upwellings now associated with continental margins would not exist. This primordial ocean, like a permanently stratified low latitude lake, would function essentially as two distinct subsystems—a warmer, wind-mixed epipelagic region and a cooler, poorly mixed, bathypelagic region. Once life began, nonconservative nutrients and reduced carbon would, as in present oceans, be removed from the surface waters by gravitational settling of organic remains.

Three implications emerge from these considerations. First, in the surface waters, evolutionary selection would favour cells with the high surface area-to-volume ratio essential for scavenging dilute nutrients. The food web, we suggest, was in gross outline comparable with that in the open oceans today[3]. Dissolved organic carbon compounds were anaerobically utilised by nanoplankton-sized saprobes and these in turn were preyed on by nano or small carnivores. With size restricted by nutrient concentrations, evolutionary selection for larger multicellular forms was absent and life remained at the unicellular level.

Second, we suggest that in light of the inverse relationship between body size and metabolic rate[4] and the lower energy yield obtained in anaerobic fermentation[5], primordial heterotrophs rapidly depleted the supply of reduced organic substrate in surface waters. On the assumptions that (1) initially the primordial ocean was 10% by weight reduced organic carbon[6]; (2) the epipelagic region extended to a depth of 100 m, (3) no reduced carbon was returned from bottom waters, and (4) the rate of organic carbon utilisation was comparable with that of heterotrophs in the oceans today, then reduced carbon reserves in surface waters could have been depleted within 250,000 yr. This suggests that in surface waters there was early evolutionary selection first for organisms able to utilise less reduced organic carbon[7], and then for photoautotrophs able to fix carbon dioxide. This argues for an early evolution of photosynthetic capability and the release of oxygen to the Earth's atmosphere and could account for the occurrence of chlorophyll in appreciable amount as early as 3×10^9 yr ago[8].

A third implication is persistence of anaerobic conditions in the bathypelagic region. Although a rate of photosynthesis comparable with that in the least productive regions of the present oceans could, in a period of 50,000 yr, yield a quantity of oxygen comparable with that in the present atmosphere, any buildup of atmospheric oxygen reserves would have little effect on the bottom waters. The continued input of reduced carbon from above, limited mixing, and the large volume of this lower region would militate against rises in oxygen concentration.

With the development of continental platforms able to obstruct horizontal ocean circulation, conditions in the primordial ocean would alter considerably. Upwellings developed along continental margins would elevate nutrient concentrations in at least limited regions of the photic zone. A consequent increase in both total energy fixed by autotrophs, and in cell size of autotrophs, would occur and in response to this latter change, the difference between carbon fixed by autotrophs and carbon utilised by autotrophs would increase. The net result would be an increase in the amount of energy available to heterotrophs and selection for metazoan forms. Platforms within 200 m of sea level may be required to initiate such upwelling[9].

At the outset selection might be for free swimming ciliated or flagellated forms structurally similar to the larval stages of most para or metazoan phyla. But with locomotor ability increasing in proportion to surface area and mass increasing in proportion to volume, a shift from planktonic to benthic modes of life would ultimately occur. Soft bodied, sessile, filter feeders, and burrowing, detrital feeders characteristic of marine benthic and neritic sediment would quickly colonise the elevated continental platforms.

Finally, evolutionary selection for large multicellular forms would come when persistent land forms emerged and regions comparable with the highly productive estuaries of today's Earth appeared. In response to the increases in nutrient concentration resulting from continental runoff, there would be selection for plants and animals able to withstand the rigours of a turbulent, near-shore environment. Large attached plants and hard-bodied animals evolved and, in a geologically brief period, colonised the newly emergent land forms.

Although the foregoing is only a sketchy outline, it is clear that if Hargraves is correct, we must rethink many ideas on biological evolution. Whereas conventional wisdom envisions life as slowly evolving until the early Cambrian and then blossoming to fill an array of previously sterile habitats, we would instead see evolution as a process rapidly generating forms to colonise each environment as it became available. The development of land masses and the evolution of life are viewed as intimately linked and the fossil record should provide dates for critical stages in Hargraves's chronology.

This vision of biological evolution in a stratified world ocean has important consequences in many other areas—among them the evolution of the terrestrial atmosphere and the origin of banded iron formation. For example, the Berkner–Marshall[10] view that Precambrian faunal development was retarded by lack of atmospheric oxygen is no longer viable. An oxygenated atmosphere nearly 3×10^9 yr before present is more consistent with fossil[11] and carbon isotope data [12,13].

Precambrian banded iron formation may represent periods of pronounced upwelling which for some reason (too brief in duration?) did not yield significant evolutionary development. Ferrous iron from upwelling waters would be precipitated on mixing with oxygenated surface waters. Seasonal variations in the intensity of coastal upwellings are not uncommon and could account for the banded character of banded iron formation. This mechanism is not grossly unlike that envisioned by others (for example ref. 14).

In conclusion it should be noted that the concept of a circumglobal sea, and the traditional view of early and persistent continents are mutually exclusive. Re-examination of data in the light of these different theories seems most appropriate.

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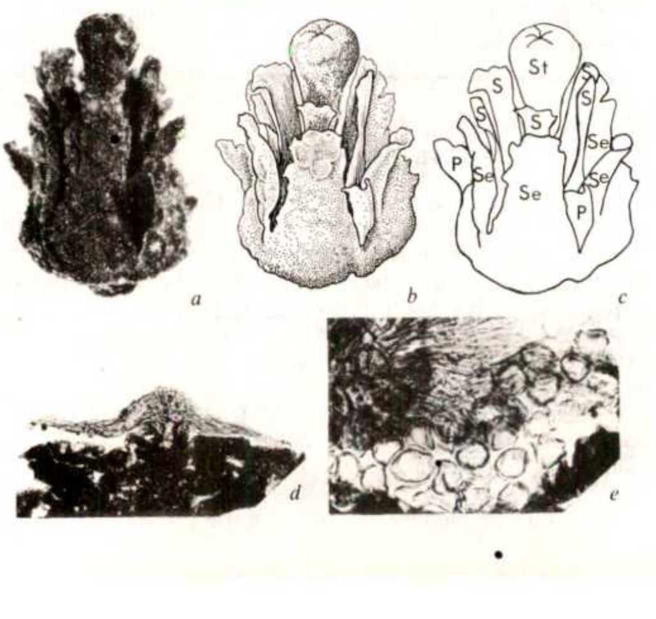
Dicotyledonous angiosperm flower from the Upper Cretaceous of Martha's Vineyard, Massachusetts

RECENT palaeobotanical studies on Cretaceous angiosperms, including those concerning the antiquity of the conduplicate carpel[1] and the first stages of the evolution of angiosperm pollen and leaf morphology[2] have contributed significantly to our knowledge of the group. However, the paucity of fossil flowers in the record[3] means that existing concepts of the nature of the early flowers of the angiosperms must be based on the comparative morphology of living forms. The discovery of the three-dimensionally preserved flower herein described, and its associated remains, is thus of considerable interest.

Upper Cretaceous plants have previously been described from ironstone concretions and sandy clays on the island of Martha's Vineyard, Massachusetts[4]. Mr Clifford Kaye of the United States Geological Survey called my attention to a more recently exposed occurrence of highly fossiliferous, dark-grey, sulphide-rich lignitic clay on the cliffs at Gay Head, at the extreme western end of the island. This clay has been dated by pollen analysis as earliest Campanian in age (Upper Cretaceous, about 75 Myr old) and is the source of the recently described gymnosperm cones *Pityostrobus kayei* and *Pseudoaraucaria arnoldii*[5]. Approximately 2,000 kg of this clay were collected, and subsequently disaggregated and sieved to concentrate the organic fraction. This was placed in hydrofluoric acid to remove adherent mineral matter, and finally washed. Examination of this material with the aid of a dissecting microscope revealed a diverse array of megaspores, gymnospermous staminate and ovulate cones, leaves and wood, and angiospermous fruits, seeds and flowers. These are all preserved as three-dimensional compactions, and retain a surprising degree of cellular detail. From this material, which forms the basis of a continuing and more detailed investigation, one particularly well preserved and distinctive flower was isolated, which is described in this preliminary report. It was carefully examined, drawn and photographed, and then embedded in paraffin by standard methods and sectioned at 10 μm intervals for examination with a compound microscope.

The 3.3-mm long flower (Fig. 1*a–c*) is apparently bisexual, possibly solitary, and borne on a short pedicel. The five sepals are basally united to form a shallow cup on which the five petals and five stamens are sequentially inserted, the stamens opposite the sepals. The calyx exceeds the corolla, but this may have been caused by the differential erosion of these two floral whorls. The gynaecium is capped by an inflated five-lobed stigma with a central depression. This five-part division is maintained in the cellular structure through the style to the ovary, suggesting the presence of five fused carpels. The compacted nature of the fossil precluded the determination of the number of locules

Fig. 1 *a*, Photograph of flower in lateral view; note the short pedicel (×12.8). *b* Drawing, looking into the flower at a slight angle (×12.8). *c*, Interpretative outline; St: stigma (the style below), S: stamen, P: petal, Se: sepal. Note that not all petals are present, and that one sepal is obscured. *d*, Median section through an umbonate, peltate trichome from the base of a stamen (×269). *e*, Tricolporoidate/tricolporate pollen (centre) and broken anther sac wall (top left) adherent to the base of one anther (bottom left and right) (×420).



and ovules present, their placentation, and the vascularisation of the individual carpels. No stylar canal is present. The laminar anthers lack whole anther sacs, but concentrations of thin-walled debris and pollen suggest that the sacs were embedded in the adaxial face of the stamen, on either side of the connective. The style, the pedicel, and the lower portions of the anthers are ornamented with umbonate, stalked, peltate trichomes which range from 120 to 150 um in diameter and are up to 20 μm thick (Fig. 1*d*). Abundant tricolporoidate to tricolporate pollen is found on the adaxial faces of the stamens (Fig. 1*e*), about the base of the gynaecium, and specifically localised on the five lobes, but not the interlobes, of the stigmatic terminus. The pollen is triangular in polar view, with the apertures borne at the angles. The exine is smooth. The average diameter of the grains is 10–12 μm. The large quantity of pollen adherent to the stigmatic surface implies that the flower was mature at the time of deposition.

The possibility that this flower represents a gymnospermous inflorescence seems remote, particularly in light of the pollen morphology, the presence of a clearly demarcated stigmatic region and the overall spatial relations of the parts. However, although the characteristics of this flower are distinctly angiospermous, their exact association does not seem to occur in any modern group, and attempts to place it in an extant angiosperm taxon have consistently failed. Indeed, the flower seems unique in its juxtaposition of putatively primitive characters (laminar stamens and its firm, resistant texture) with more advanced ones (connate carpels and styles, tricolporoidate/tricolporate pollen, and the small, presumably reduced, number of floral parts). This association of characters is perhaps most notable in that it is advanced beyond those postulated for the most primitive angiosperms[6,7], and thus reflects a "minimum" level of evolution achieved by the group by the Campanian. It is tempting to draw parallels between this fossil and various modern taxa. However, to do so would be to force it into the existing preconceived notions of angiosperm evolution, which are primarily based on the living flora.

It is thus deemed more productive to use the approach pioneered by Doyle and Hickey[2] with respect to leaves and pollen, in which the specimen is compared to other fossil material collected from older and younger horizons, as well as to material from the same horizon. The resulting "relative" taxonomy permits the recognition of trends within the fossil material which might otherwise be obscured. Continued investigation of the floral remains from this particular locality may reveal the presence of a distinctive grade of evolution, serving to elucidate the observed grouping of characters within this fossil as well as to indicate the broader trend of evolution within the dicotyledons at this time in their history.

I thank Mr Clifford Kaye for calling my attention to this locality, and for his interest and support; the selectmen of Gay Head, Massachusetts, for permission to collect at Gay Head cliffs; Professors Carroll Wood and Richard Howard of the Arnold Arboretum of Harvard University for taxonomic expertise, and Professors Elso S. Barghoorn and Rolla Tryon of Harvard University and Dr Leo J. Hickey of the Smithsonian Institution for comments on the manuscript. Figure 1*b* was drawn by Ms Karen Velmure, Arnold Arboretum. The research was supported in part by a grant from the Fernald Fund of the Gray Herbarium of Harvard University.

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Effects of plant type, size of geographical range and taxonomic isolation on number of insect species associated with British plants

IN this communication we analyse the insects associated with higher plants other than trees, which have already received attention[1–5]. We are concerned with two basic problems. First, we ask how various types of higher plants differ in the numbers of species of insects which they support; we do this by comparing standard species–area curves[1,3,6,7] for five different sorts of plants. Then we consider how the "taxonomic isolation" of a plant, measured simply as the number of related species in the same genus and geographical region, might also influence the total number of insect species to be found on it. We find that while plant type and geographical range have a considerable effect, taxonomic isolation does not, except in the case of monocotyledonous herbs.

Our data come from the accounts of individual plant species given in the *Biological Flora of the British Isles*, published in the *Journal of Ecology* between volumes 36 (1948) and 61 (1973). We excluded the grasses (Graminae) because the insect data appeared to be particularly unreliable. (The entomological literature on which the accounts are based frequently records "grass" as a food plant, rather than the species of grass involved.) An arbitrary series of decisions, inevitably introducing some error, was also made about the accounts which listed no insect species for particular plants. Where an author used a term like "none observed" we included the record as a zero; where he used a term like "no information" we excluded it.

We are aware that many of the *Biological Flora* accounts are incomplete, but since they are compiled by numerous different authors over a long period this seems merely to have increased the variance in the data, rather than introduced systematic errors. It does mean, however, that our statements about the insects associated with different types of plants are based on relative and not absolute comparisons. The present geographical ranges of each of the plants within the British Isles, excluding Ireland (where there are grounds for believing that both the plant distribution and insect data may be less reliable) and the sites of known introductions, were obtained from the *Atlas of British Flora*[8]. The *Atlas* gives the distribution on each species on a presence or absence basis using grid squares of 10 km × 10 km ("10-km squares").

Within the *Biological Flora* data, five distinct groups of plants could be recognised (embracing the families and species listed in Table 1). (1) A group of 27 herbaceous, perennial (or in two cases perennial/biennial) dicotyledons. (The addition of a perennial fern with similar harbaceous growth form, and three creeping perennial but woody dicots made no difference to any of the conclusions listed below; these four species are distinguished in Table 1.) (2) a group of 10 perennial dicotyledenous woody bushes and small shrubs. (3) A group of 16 predominantly annual dicotyledons. Eight of these species are common agricultural weeds (seven annuals and one perennial). The other eight species are all annuals (or in one case annual/biennial) from various natural open or disturbed habitats, and "waste places". (4) A group of 14 monocotyledenous herbs. (5) A small group of four aquatic perennial dicots.

For convenience, these five categories will be referred to as "perennial herbs", "woody shrubs", "weeds and other annuals",

Table 1 Plant families and species in each of five groups recognised in our analyses

(1) *Perennial herbs* (Species in brackets also assigned to this category are a fern, and three creeping woody dicots.)	
Boraginaceae	*Myosotis alpestris*
Caryophyllaceae	*Silene nutans*
Cistaceae	(*Helianthemum chamaecistus*)
Compositae	*Chrysanthemum leucanthemum* *Cirsium acaulon* *Senecio jacobaea* *Succisa pratensis*
Cruciferae	*Draba aizoides*
Gentianaceae	*Gentiana verna*
Labiatae	*Teucrium scorodonia* (*Thymus* spp.) (*drucei* distribution data used)
Lobeliaceae	*Lobelia urens*
Papaveraceae	*Glaucium flavum*
Papilionaceae	*Hippocrepis comosa* *Lathyrus japonicus*
Plantaginaceae	*Plantago lanceolata* *P. major* *P. media*
Plumbaginaceae	*Limonium vulgare*
Polemoniaceae	*Polemonium caeruleum*
Polygonaceae	*Rumex crispus* *R. obtusifolius*
Polypodiaceae	(*Dryopteris villarii*)
Ranunculaceae	*Ranunculus acris* *R. bulbosus* *R. repens*
Rosaceae	(*Dryas octopetala*) *Sibbaldia procumbens* *Rubus chamaemorus*
Saxifragaceae	*Saxifraga hypnoides*
Urticaceae	*Urtica dioica*
(2) Woody shrubs	
Chenopodiaceae	*Halimione portulacoides*
Elaeagnaceae	*Hippophaë rhamnoides*
Empetraceae	*Empetrum nigrum*
Ericaceae	*Calluna vulgaris* *Erica cinerea* *E. tetralix* *Phyllodoce caerulea* *Vaccinium myrtillus* *V. vitis-idaea*
Rosaceae	*Potentilla fruticosa*
(3) Weeds and other annuals	
Balsaminaceae	*Impatiens parviflora*
Caryophyllaceae	*Corrigiola litoralis* *Spergula arvensis*
Chenopodiaceae	*Chenopodium album*
Compositae	*Anthemis arvensis* *A. cotula* *Sonchus oleraceus/asper*
Convolvulaceae	*Cuscuta epithymum* *C. europaea*
Cruciferae	*Cardaria draba* *Draba muralis* *Hornungia petraea* *Sinapis arvensis*
Papaveraceae	*Papaver rhoeas*
Plantaginaceae	*Plantago coronopus*
Scrophulariaceae	*Melampyrum cristatum*
(4) Monocots	
Amaryllidaceae	*Narcissus pseudonarcissus*
Araceae	*Arum maculatum*
Cyperaceae	*Carex flacca* *Eleocharis palustris* *Eriophorum angustifolium* *E. vaginatum* *Schoenus nigricans*
Juncaceae	*Juncus acutus* *J. squarrosus*
Liliaceae	*Allium ursinum* *Colchicum autumnale* *Endymion non-scriptus*
Orchidaceae	*Orchis purpurea*
Sparganiaceae	*Sparganium erectum*
(5) Aquatic dicots	
Loleliaceae	*Lobelia dortmanna*
Menyanthaceae	*Menyanthes trifloliata*
Nymphaeceae	*Nuphar* spp. (*lutea* distribution data used) *Nymphaea alba*

"monocots" and "aquatic dicots". Figure 1 shows their species–area relationships. S is the number of species of insect associated with each plant species, and A is the geographical range of that plant within the British Isles in 10-km squares. Because the axes are logarithmic, $S+1$ was used so that we could include those species of plants from which no insects were recorded.

The equations are:

Perennial herbs (Fig. 1*a*)

$$\ln[S+1] = 0.54 \ln A - 0.95 \qquad (1)$$
$$r^2 = 0.71;\ F_{1,29} = 69.57;\ P < 0.001$$

Woody shrubs (Fig. 1*b*)

$$\ln[S+1] = 0.45 \ln A + 0.005 \qquad (2)$$
$$r^2 = 0.85;\ F_{1,8} = 45.51;\ P < 0.001$$

Weeds and other annuals (Fig. 1*c*)

$$\ln[S+1] = 0.47 \ln A - 1.33 \qquad (3)$$
$$r^2 = 0.59;\ F_{1,14} = 20.32;\ 0.005 > P > 0.001$$

Monocots (Fig. 1*d*)

$$\ln[S+1] = 0.39 \ln A - 1.60 \qquad (4)$$
$$r^2 = 0.51;\ F_{1,12} = 12.64;\ 0.005 > P\ 0.001$$

No attempt was made to fit a regression line to the four points available for the aquatic dicots. They are illustrated as Fig. 1*a* for completeness.

Table 2 summarises a statistical comparison of the four regressions. None of them differs from any of the others in either residual variance or slope; they differ only in intercept. Each of the group of plants has a quite characteristic number of insect species associated with it, for a particular size of geographical range, in the sequence woody shrubs = perennial herbs > weeds and other annuals > monocots. Although the regression line for the woody shrubs lies above that for perennial herbs, the difference is not statistically significant. On the limited data available, the aquatic dicots seem to occupy a position somewhere between the weeds and other annuals, and the monocots.

We next tested the hypothesis[9-11] that taxonomic isolation reduces the number of species of insects associated with a plant, on the grounds that the "exchange" of herbivores between closely related plant species will be easier than "exchange" between unrelated species[11]. This assumes that, in general, closely related plants are biochemically and structurally more similar than less closely related ones, which does not seem unreasonable[12,13]. "Exchange" is used here to denote the aquisition of a herbivore guild on an evolutionary timescale. Figure 2 shows the residuals (R) derived from the appropriate species–area curves shown in Fig. 1 (equations (1)–(4)), plotted against the number of plant species in the genus (G) within the British Isles. (The number of species in the genus on a worldwide basis[14] yielded no sensible patterns.) Log G provided the best fits to these data, giving for:

Perennial herbs (Fig. 2*a*)

$$R = 0.19 \ln G - 0.32 \qquad (5)$$
$$r^2 = 0.07;\ F_{1,29} = 2.07;\ 0.25 > P > 0.1 \text{ n.s.}$$

Monocots (Fig. 2*d*)

$$R = 0.30 \ln G - 0.43 \qquad (6)$$
$$r^2 = 0.46;\ F_{1,12} = 10.28;\ 0.01 > P > 0.005$$

Only equation (6) is statistically significant. There was no relationship between R and G for either the woody shrubs (Fig. 2*b*) or the weeds and other annuals (Fig. 2*c*). The perennial herbs (equation (5)) show signs of the positive relationship predicted by the hypothesis, but this is not statistically significant.

If we view the number of species on a particular plant as a balance between colonisation and extinction, then geographically more widespread plants have a larger number of species associated with them because they are more likely to be found

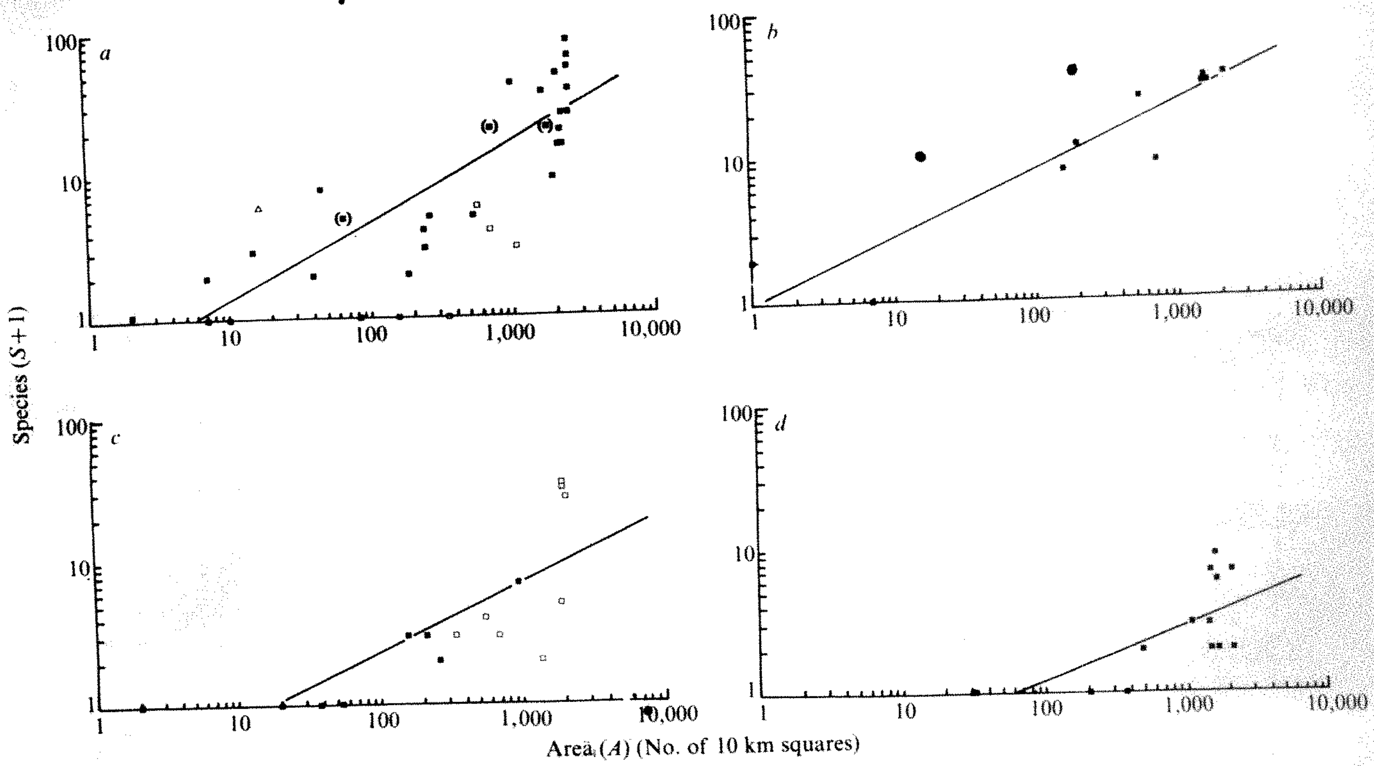

Fig. 1 Insect species associated with various groups of British plants, as a function of the size of the plant's geographical range (measured in terms of the number of 10-km squares occupied by the plant in the *Atlas of the British Flora*[8]). *a*, Perennial herbs. ■, Terrestrial herbaceous dicots; (■), creeping woody dicots; △, perennial fern; □, aquatic perennial dicots (not included in regression line). *b*, Woody shrubs. *c*, Weeds and other annuals. ■, Weeds; □, other annuals. *d*, Monocots (excluding grasses).

and colonised given sufficient time[1,3,4,9,11]. In Feeny's terminology[12], they are more "apparent". Unpredictability in time and space (weeds and other annuals) reduces "apparency" and increases the probability of extinctions; so does rarity. Hence the equilibrium number of species on rare and/or unpredicatable plants is reduced. Why monocots should have even lower equilibria is less obvious. We are inclined to the view that this is a function of their "architecture" with woody shrubs, perennial herbs and monocots forming a series of decreasing structural complexity. Structurally more complex plants permit greater niche diversification (both for competitor avoidance, and the avoidance of your neighbours' predators or parasites[15,16]) and hence have a bigger equilibrium species pool. Trees are even more structurally complex, and visual inspection of Strong's data for British trees[3] suggests higher numbers of associated species, area for area, than woody shrubs or perennial herbs. (A direct comparison is impossible, because Strong's equation does not fit his own results: also he included, and we excluded, Ireland from the plant-area measurements.) The complete series therefore appears to be trees > woody shrubs = perennial herbs > weeds and other annuals > monocots.

The paucity of insects on aquatic dicots (which fall in this series between weeds and other annuals, and monocots) is well known[17] and presumably reflects low colonisation (or high extinction) rates; why this should be however, is very uncertain.

Monocots excepted, there is no evidence to suggest that taxonomic isolation influences the number of species of insect associated with a particular plant. This may merely reflect the inadequacy of our data, our way of testing for taxonomic isolation, or some fundamental difference between monocots and other plants. For example, the total "pool" of insects adapted to feed on monocots may be so small that it has significantly impaired the probability that a taxonomically

Table 2 Statistical comparison of regressions 1–4

	Woody shrubs (2)	Weeds and other annuals (3)	Monocots (4)
Perennial herbs (1)	$V{:}F_{29,8} = 2.37$ n.s. $b{:}F_{1,37} = 0.65$ n.s. $c{:}F_{1,38} = 2.79$ $0.25 > P > 0.1$ n.s.	$V{:}F_{14,29} = 1.01$ n.s. $b{:}F_{1,43} = 0.34$ n.s. $c{:}F_{1,44} = 10.41$ $0.005 > P > 0.001$	$V{:}F_{29,12} = 1.89$ n.s. $b{:}F_{1,41} = 0.96$ n.s. $c{:}F_{1,42} = 44.30$ $P < 0.001$
Woody shrubs (2)		$V{:}F_{14,8} = 2.39$ n.s. $b{:}F_{1,22} = 0.02$ n.s. $c{:}F_{1,23} = 19.20$ $P < 0.001$	$V{:}F_{12,8} = 1.25$ n.s. $b{:}F_{1,20} = 0.21$ n.s. $c{:}F_{1,21} = 72.6$ $P < 0.001$
Weeds and other annuals (3)			$V{:}F_{14,12} = 1.91$ n.s. $b{:}F_{1,26} = 0.23$ n.s. $c{:}F_{1,27} = 8.51$ $0.01 > P > 0.005$

V, comparison of residual variances; *b*, comparison of slopes; *c*, comparison of intercepts.

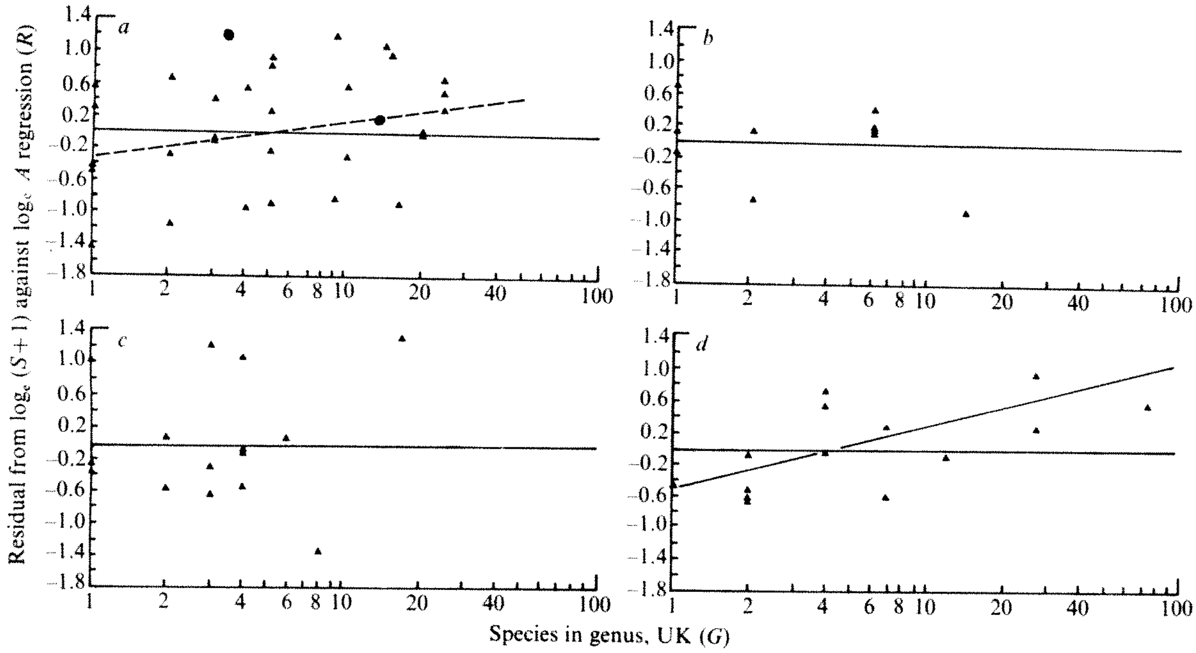

Fig. 2 The residuals from the fitted regression lines in Fig. 1, plotted against the number of species of plants within each genus in the United Kingdom. (The number of plant species in each genus is a measure of "taxonomic isolation".) *a*, Perennial herbs; *b*, woody shrubs; *c*, weeds and other annuals; *d*, monocots. Only the monocots (*d*) show a significant relationship.

isolated member of this group will be colonised over evolutionary time. With a larger total "pool" (like that on perennial herbs) the effects of taxonomic isolation have been lost. This viewpoint obviously demands that the insects which exploit dicots have a negligible probability of contributing to the monocot "pool". It also raises some important questions about what is meant by an equilibrium assemblage[3-5], and the time scale over which this should be measured. These problems are beyond the scope of the present note.

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Evidence, using radioactive phosphorus, of interspecific food exchange in ants

MEMBERS of many ant species exchange fluid foods within the colony[1-4]. Similar exchange between social symbionts, living as "slaves"[5] or "guests"[6] and their hosts, is also documented[1]. However, in non-symbiotic species, found in the same habitat or in distinctly different habitats, this phenomenon is usually overlooked. We document here that the food-transporting worker ant of one species offered carbohydrate food to the aggressive worker ants of another as a means of suppressing aggression in the latter, as in appeasement displays in higher animals. The workers of the aggressive red fire ant species, *Solenopsis invicta*, introduced into the southern United States in the 1930s but comparatively recently named[7], were invariably offered regurgitated fluids by indigenous species *S. geminata* and *Pheidole dentata* which had been fed earlier with ^{32}P-labelled 20% sucrose solution.

In 1.5 cm diameter, 1.5 cm wide glass cells, where the donors and aggressors could encounter each other quite often, a single donor worker offered the regurgitate to four aggressors within 15 s of their first encounter, and all the aggressors were offered food within 20–30 min. Such a food offer was made regardless of the aggressor's caste. Both the *Solenopsis* species are polymorphic and *P. dentata* is dimorphic. In addition to major and minor castes, the polymorphic species contained a gradation of medium-sized workers. Immediately following antennal contact between the donor species and the aggressor species, both opened their mandibles: the aggressor apparently to alarm its sister workers and to seize the body parts of the donor, whereas the donor acted thus to regurgitate a large drop of fluid between its mandibles. As the alien species became more aggressive, seizing the donor, stinging, and so on, the food-offering responses of the donor also intensified: the donor contacted the aggressor's antennal bases with its vibrating antennae and oriented the food droplet towards the aggressor (Fig. 1). When the donor was kept with workers of its own species, the workers contacted its antennal bases with theirs, in food solicitation. In some cases, food was offered only after the solicitors groomed the donor with their mouth parts in a ritualised fashion from legs up to the cephalic ventrum. The contrast in food-offering behaviour by one donor worker towards its own sister workers, and the aggressive workers of an alien species, suggested

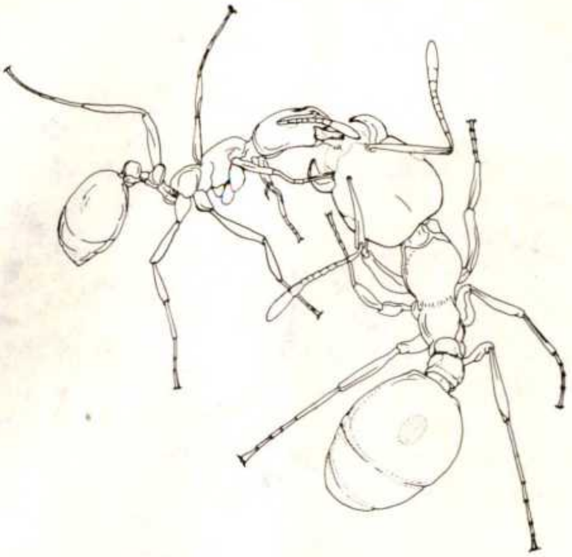

Fig. 1 Unusual interspecific trophallaxis between a donor of *Solenopsis invicta* (major worker with hatched spot on abdomen) and an aggressive solicitor minor worker of *S. geminata* drawn from actual photograph. The "appeased" solicitor touched the donor's mandibles with its forelegs and held antennae near head while feeding. The donor held its antennae in a W-shape and moved them to and fro while being solicited. The donors are fed on $^3H^{32}PO_4$ (specific activity 1.8 mCi ml^{-1}) in 150 g Gerber baby food jars for 2 h. They were made less mobile by exposure to CO_2 for 5–10 s and were surface decontaminated by passing through inactive NaH_2PO_4 solution[1]. The radioactivity was measured by using a GM-end-window (thickness 1.2–1.4 mg cm^{-2}) counting tube in conjunction with a Berthold Scaler Timer BF 22/25.

that in the latter case, the readiness of the donor to offer food reduced the aggression in the aggressor species.

In the southern United States, where *S. invicta* is regarded as a nuisance, it subdued under laboratory conditions the colonies of over 30 species, comprising six subfamilies. It killed twice its number of *S. geminata* and four times its number of *P. dentata* when their workers confronted each other in large numbers[8]. When the workers of *S. geminata* and *P. dentata* were presented to each other in the ratio of four aggressors to one donor, and *S. invicta* was a donor species, food transfer occurred. No sooner had the donor offered its alimentary fluid to the aggressors, than their aggressive frenzy diminished and the food was accepted. The food was aggressively solicited a number of times within the short period of 30 min. The workers that did not accept food were especially aggressive, that is they elicited mandibular attack, stinging, and so on. The alien workers accepted food and groomed themselves. In the laboratory, the donor showed escape behaviour by crawling on the glass cell. In nature, the aggressor's pause to eat and groom itself could make the donor's escape possible.

Variations in the distribution of food from major or minor caste donors to similar or dissimilar castes of aggressive solicitors, in all the three species, may be due to the differential aggressive element present in individuals or castes, and the individual's own food reserve. This explanation may hold true for less frequent food transfer from the workers of *S. invicta* to the workers of *S. geminata* or *P. dentata*, that is the aggressive element in *S. invicta* dominated that in the other two species (Table 1). An overt form of defensive behaviour in the major workers of *S. geminata* and *P. dentata* with the use of a novel alarm-recruitment system has been documented[9]. During the colony confrontation studies between *S. invicta* and two species of *Pheidole*, the guarding workers of *P. dentata* and *P. morrisi* exchanged food with the nest-invading *S. invicta* workers and delayed their invasion by 20–30 min (A. P. Bhatkar, unpublished).

Monomorphic species where workers exchange fluid food within the colony, such as a species of subgenus *Diplorhoptrum*, *S. pergandei*, and *Monomorium pharaonis*, escaped the attacks of *S. invicta* by slipping through their wide open mandibles. The workers of both these species are approximately half the size of the smallest *S. invicta* (smallest *S. invicta* 3.2 mm, largest 7.5 mm). During prolonged exposures (20 min–60 min), however, *S. invicta* obtained food by piercing the soft cuticle of slow moving, less aggressive donors. Similarly, otherwise aggressive woodland species with monomorphic workers (1.5 times as large as the largest *S. invicta* worker), *Aphaenogaster lamellidens*, fell victim to *S. invicta* due to diminished aggression and mobility in the donors. We may add that intracolonial exchange of fluid foods was absent in *A. lamellidens*.

Exchange of alimentary liquids (solutions, emulsions and suspensions) between colony members, including social symbionts, is usually termed "trophallaxis"[9]. If the scope of this definition is appropriately widened, the occurrence of food transfer in nonsymbiotic species of the type described above could very well be termed "interspecific trophallaxis". Interspecific associations in the ant colonies have one common denominator which is that ultimately they are all trophic in relation. The development of all forms of social symbioses among ant colonies, from plesiobiosis (nesting in proximity with little or no communication), xenobiosis (food sharing, keeping separate broods), to dulosis (one species robbing the brood of another and increasing its common worker force) and inquilinism (one species passing its

Table 1 Food exchange in different ant species expressed in terms of transfer of radioactivity from donor to recipient workers

Donor species and caste	Recipient species and caste	Radioactive ants per ants exposed in all replicates	c.p.m. radioactivity per recipient mean	s.d.
S. geminata	*S. invicta*			
minor	minor	16/16	62.88	136.50
minor	major	16/16	21.00	16.13
major	minor	8/16	9.69	20.77
major	major	14/16	254.75	589.49
P. dentata				
minor	minor	11/16	105.75	169.61
minor	major	6/12	301.16	547.49
major	minor	13/16	310.19	466.76
major	major	4/12	175.00	434.63
S. invicta	*S. geminata*			
minor	minor	9/16	45.63	169.61
S. invicta	*P. dentata*			
minor	major	5/16	7.75	9.37

Food distribution from donor to recipients, and secondarily, among recipients was not normal. High s.d. was due to some workers receiving food repeatedly while the others in small amount. Experiments were conducted at 28 °C, replicated three or four times, and the exposure of one donor to four aggressive solicitors in each replicate was 1–5 h.

life cycle at the cost of other) seem to have interspecific food exchange as a driving force in their evolution. Such a phenomenon among phylogenetically and ecologically separate species is possibly an adjustment against competition even when they come together by accident, such as the red imported fire ant from Brazil and the Florida Myrmicinae, described here.

This research was conducted at the University of Florida, Gainesville, in 1972 when the authors were graduate student and visiting professor respectively.

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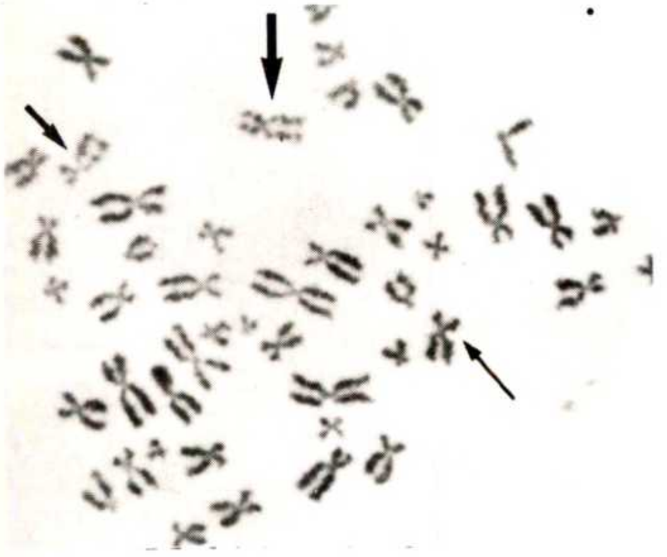
Presumptive evidence of two active X chromosomes in somatic cells of a human female

IN a female child with various morphological abnormalities and partial translocation trisomy X—karyotype: 46,XX,−22, +der(22),t(X;22)(q12;p11)mat—we have found evidence of two euchromatic X chromosomes in about 15% of metaphases from lymphocyte cultures. We believe this means that in these cells there were two active X chromosomes.

After treatment with BUdR (200 μg ml^{-1}) during the final 7 h of culture different patterns of decondensation were observed in individual metaphases. In 50% of cells an entire X and the long arm of the X/22 translocation chromosome, der(22), were decondensed (Fig. 1). In a smaller proportion of mitoses, however, the two normal Xs were of equal length, showing a reversed banding pattern, whereas the long arm of der(22) was highly decondensed (Fig. 2). In another cell line the der(22) and one whole X showed normal condensation, the other X being decondensed (Fig. 3). The rest of the karyotypes were uninformative.

Fig. 1 Type 3 cell. The thin arrow shows euchromatic X; the thick arrow, heterochromatic X, and the medium arrow, heterochromatic long arm of der(22) (verified by Q fluorescence).



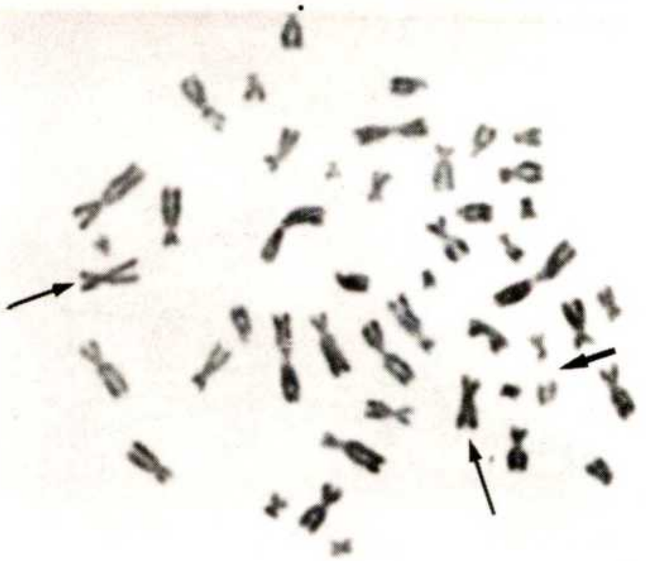


Fig. 2 Type 1 cell. The thin arrow shows euchromatic Xs, and the thick arrow shows heterochromatic long arm of der(22) (verified by Q fluorescence).

After treatment of blood cultures with ^{3}H-thymidine (1 μCi ml^{-1}) for the final 7 h of culture three different late replication patterns were observed. (1) In the majority of suitable metaphases one entire X and the der(22) were heavily labelled; (2) in a smaller proportion of cells only the long arm of the der(22) was late labelling, the two normal Xs showing only a few grains. (3) In a few mitoses one X was late replicating, whereas the other X and the der(22) showed an early pattern.

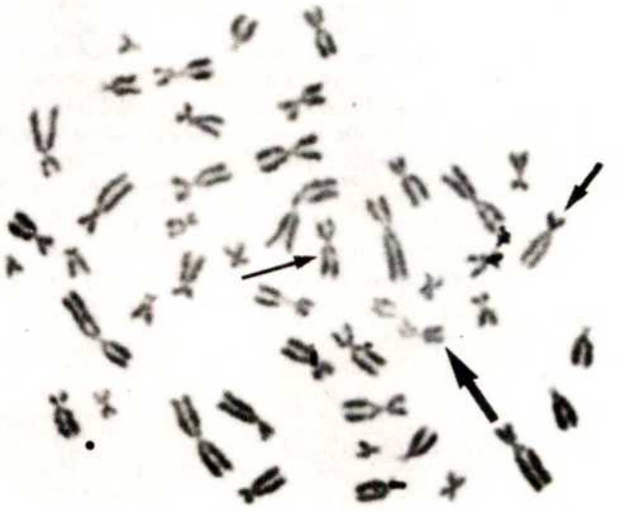


Fig. 3 Type 2 cell. The thin arrow shows euchromatic X; the medium arrow, euchromatic der(22), and the thick arrow, heterochromatic X (verified by Q fluorescence).

Percentages and sample sizes are summarised in Table 1. The different percentages of type 2 and type 3 cells found with BUdR and autoradiography respectively can probably be explained by the small sample size. (The uninformative observations are not entered in Table 1. They account for the differences between summed percentages and 100%.)

Four different sex chromatin patterns were found in buccal mucosa cells, hair root cells and fibroblasts (Fig. 4).

Table 1 Patterns of labelling

	Blood cultures		Observations			Interpretation
	Decondensation	Late labelling	Sex chromatin			
Cell type	BUdR	^{3}H-thymidine	Hair roots	Buccal mucosa	Fibroblasts*	Active X chromosomes†
1	der(22) 12,5%	der(22) 17%	S 14%	S 10%	S 16%	XX
2	X 25%	X 7%	N 26%	N 35%	N 32%	XXq
3	X der(22) 50%	X der(22) 76%	N+S 4%	N+S 11%	N+S 10%	X
4	XX 0%	XX 0%	2N 4%	2N 16%	2N 10%	(Xq)‡
Total cells examined	32	42	250	500	600	

N, normal sized Barr body; S, small Barr body.
* Courtesy of Dr W. Vogel, Institute of Human Genetics; Freiburg im Breisgau.
† Xq, Xq12 → Xqter.
‡ See text.

There were single normal sized Barr bodies, double Barr bodies of equal size, double bodies of different size and single small bodies. The latter are thought to be derived from the der(22) chromosome, consisting of Xq only, and the normal sized sex chromatin bodies are thought to be derived from the morphologically normal X. Two equal sized Barr bodies would then suggest the presence of cells with two equal sized heterochromatic Xs. This type of cell (type 4, Table 1), however, was never identified in blood cultures with either BUdR or ^{3}H-thymidine. The nuclei with two Barr bodies of apparently equal size were probably cells with one entire X and the der(22) inactivated, the difference in size between the two not being detectable in every interphase nucleus. The size of the Barr bodies in cases of Xq− or i(Xp) as well as Xp− has been reported sometimes to appear normal, for example as reported by Van den Berghe *et al.*[1] and Atkins *et al.*[2]. The most striking discrepancy between metaphases of cultured blood cells and sex chromatin findings in interphase nuclei was the relative paucity of nuclei with double bodies, especially in hair root cells and fibroblasts, compared with 50% and 76% of blood cells with one X and the der(22) inactivated (type 3, Table 1). It is well known, however, that in proven cases of triple X syndrome only a certain percentage of cells shows double Barr bodies, just as in normal females only between 20% and 50% of interphase nuclei are sex chromatin positive, varying according to the tissue examined and the method used. There is very good agreement with respect to cells with two active Xs (type 1, Table 1) and fair agreement for cells of types 1 and 2 taken together, allowing for the fact that some of the cells with one normal sized body are probably cells of type 3 with one X and the der(22) inactivated. It is also possible that the four cell types are present in different ratios in different tissues. Type 4 cells (Table 1) probably do not exist in lymphocytes. Whether they exist in other tissues or whether the recorded double Barr bodies of equal, normal size were Barr bodies derived from structurally different chromosomes (X and der(22)) corresponding to type 3 cells remains to be shown.

The mother of the patient had normal sex chromatin counts. She had the same translocation chromosome der(22) in addition to one normal and one deleted X, consisting of Xp, centromere and proximal Xq—karyotype: 46,X,t(X;22)(q12:p11). She had a normal phenotype.

At 6 months the child had failed to grow and gain weight and had deformities of head and face with low set ears, small lower jaw, upward slant to the eyes as in mongoloids, beaked nose, flat bridge of the nose, epicanthal folds, congenital heart disease and hypertonus of muscles. This phenotype cannot be explained solely by the partial trisomy X. It could be explained by monosomy 22 through spread of inactivation on the translocated 22. But the translocated 22 never showed any elongation or fuzziness or decondensation indicating inactivation (Figs 1 and 2) as shown for two X/autosome translocations by the BUdR method[3] and autoradiographically for an X/21 translocation[4]. In sufficiently stained metaphases with straight (neither bent nor twisted) chromosomes no label was seen over the short arm of the der(22) chromosome (Fig. 5) in neither cell type. If this should prove to be the case in other tissues, the reason for the malformations of the child must be sought in the gross genetic unbalance caused by noninactivation of

Fig. 4 Barr bodies in fibroblasts. *a*, Type 1, small Barr body; *b*, type 2, normal Barr body; *c*, type 3, small plus normal Barr bodies; *d*, type 4, two normal Barr bodies.

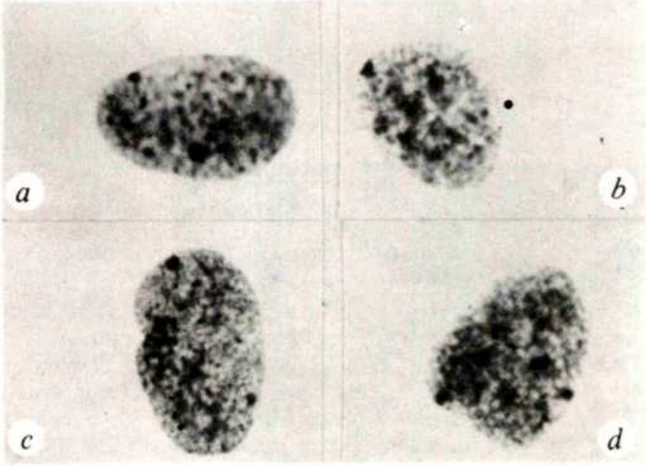
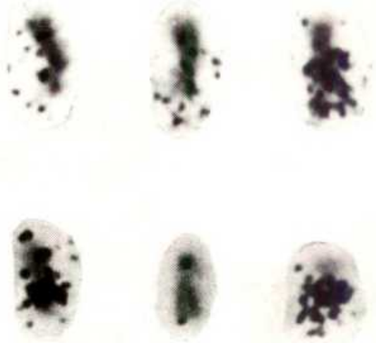


Fig. 5 X chromosomes and der(22) (verified by trypsin Giemsa banding) from two metaphases (type 1 upper, and type 3 lower) labelled with ^{3}H-thymidine. Note unlabelled short arm of der(22).



all X material in excess of one whole X, provided that we can equate decondensation and late replication with genetic inactivity.

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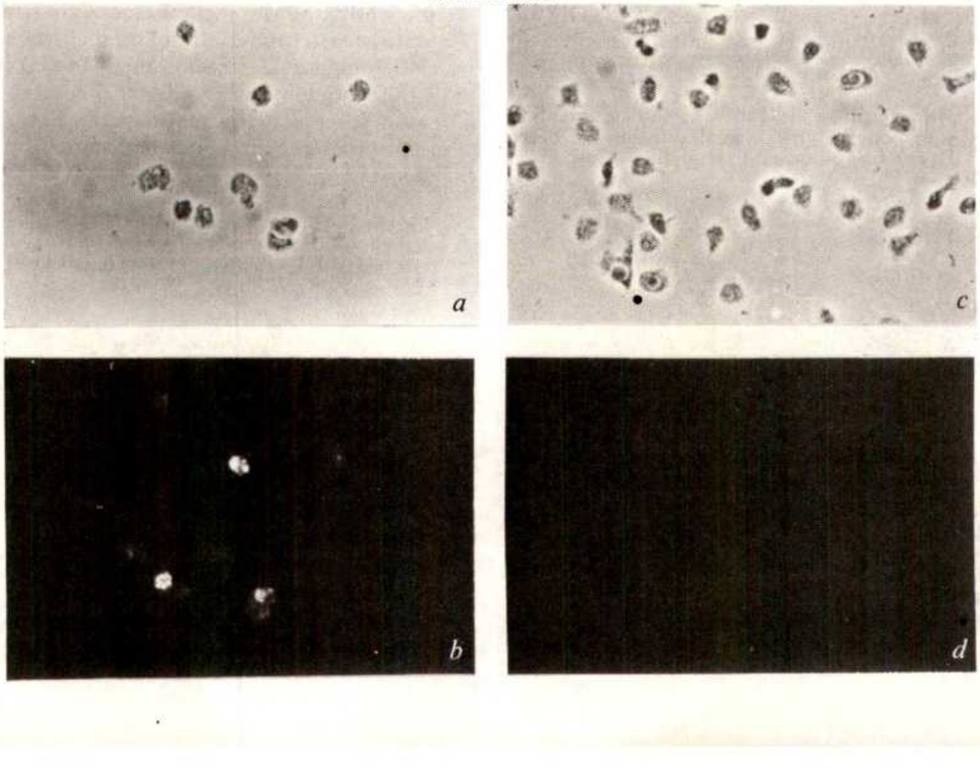
Pattern formation in the absence of polarity in *Dictyostelium discoideum*

MANY theories have been advanced as to how the spore–stalk pattern of the slime mould *Dictyostelium discoideum* is established. Without exception these envisage the polarity of the grex stage of the life cycle as a key factor in specifying the pattern. One group of theories[2–4] sees the combined influence of polarity and the grex tip as signalling positional information[5,6] which determines that the front cells differentiate into prestalk cells and the back cells into prespore cells. An alternative view is that pattern formation occurs by sorting out within the grex of cell types which are predetermined at an earlier stage of the life cycle[7–11]. Grex polarity is also a key factor in one suggested mechanism for sorting-out in that prestalk cells are seen as moving faster than prespore cells and therefore coming to the front of the migrating grex[8,12]. Because all these thories clearly suggest that pattern formation ought not to occur in circumstances in which the cell mass is unpolarised, we asked the question: 'Is polarity necessary for differentiation and pattern formation in *D. discoideum*?' We have found that a prestalk–prespore pattern very similar to that seen in the normal life cycle forms in spherical aggregates which, before pattern formation, have no detectable morphological polarity. We also describe the formation of unusual cyst-like structures from patterned spherical aggregates.

Spherical aggregates were formed by allowing axenically grown log phase cells of *D. discoideum* (strain AX-2) to adhere together in rotated suspension in phosphate buffer[13]. In such conditions cells begin adhering immediately, eventually forming spherical aggregates of about 100 μm in diameter. The cells became aggregation competent after approximately 8 h (ref. 14).

The first question we asked was whether differentiation of prespore cells would take place in such aggregates. This was tested by collecting aggregates at different times, dissociating them into single cells which were allowed to stick to a slide, and fixing and staining these with a fluorescein-labelled anti-spore serum prepared against the spores of *D. mucoroides* (modified from the method of Takeuchi), ref. 15 and D.F., and D.R.G., in preparation), (Fig. 1*a* and *b*). We found that specifically staining intracellular vesicles characteristic of prespore cells began to appear in a proportion of the cells between 12 and 13 h of shaking. By 17–18 h the vesicles stained more intensely and

Fig. 1 ×1,000. Cells dissociated from 18-h aggregates observed under phase-contrast (*a*) and under ultraviolet light (*b*), having been stained with specific anti-spore serum labelled with fluorescein isothiocyanate (FITC). Some of the cells show the development of specific prespore vesicles (PSVs). The same cells shaken more vigorously (*c*) remain single and on staining (*d*) show no specific reaction. *Dictyostelium discoideum* vegetative cells (strain Ax-2) were harvested from axenic growth medium[28], washed three times in cold distilled water, and resuspended in 0.0167 M phosphate buffer (*p*H 6.0) at a density of 10^6 cells ml^{-1}. Aliquots (4 ml) of the cell suspension were put into 25-ml flasks on a rotary shaker at 140 r.p.m. (radius of rotation ¼ inch: temperature 22 °C). The aggregates formed at 18 h were dissociated into single cells by trituration in cold distilled water. The single cells were suspended in standard salt solution[29] at 2×10^5 cells ml^{-1} and allowed to settle over and adhere to glass coverslips. Fixation and staining of cells with specific antispore serum was carried out as previously described[15]. For fluorescent microscopy cells were observed on a Zeiss Universal microscope, fitted with an HBO 200 mercury arc lamp, BG 38 and BG 12 exciter filters and Zeiss barrier filters 50 and 44. Cells treated with a control non-immune serum showed no specific staining. Cells were kept single and prevented from forming aggregates by shaking at 260 r.p.m. These cells were tested for aggregation competence at 8 h by allowing them to settle on to coverslips and looking for the formation of characteristic elongated shapes and development of end-to-end chain-like contacts between cells.



were present in a greater proportion of the cells. These vesicles seemed identical to the prespore vesicles (PSV) which have been identified in late aggregates and grexes when cells are allowed to develop in normal conditions on a surface (refs 15, 16 and D.F., and D.R.G., in preparation). The time of appearance of prespore cells in spherical aggregates was approximately the same (that is, 12 h after the end of the vegetative phase and 4 h after chemotactic aggregation) as in cells undergoing normal development (D.F., and D.R.G., in preparation).

When vegetative cells were kept single in suspension by vigorous shaking, they became aggregation competent after about 8 h even though they did not form aggregates. Would such cells also differentiate into prespore cells? Even after 36 h of shaking in suspension single cells showed no sign of staining with specific anti-spore serum though they remained viable (Fig. 1*c* and *d*). It seems that single cells are capable of differentiation to the aggregation stage but cell contact is an essential prerequisite for further differentiation into prespore cells.

Since morphological polarity of aggregates was not essential for prespore differentiation, we proceeded to ask whether the formation of a prespore : prestalk pattern could also occur in the absence of polarity. Fluorescent antibody staining of sections through 18 h aggregates showed no obvious patterning of the arrangement of stained and unstained cells (Fig. 2*a*). By 24 h, however, there was a clear spatial separation of the two cell types (Fig. 2*b*). Examination of many randomly sectioned aggregates showed the structure to be a mass of stained prespore cells partially surrounded by a cap of unstained cells. When 24-h aggregates were plated on to an agar surface they developed directly into normal fruiting bodies.

Because differentiation and pattern formation could proceed to a considerable extent within spherical aggregates by 24 h, shaking was continued for longer periods to see whether differentiation into mature spores and stalk would occur. We found that after 4 d of shaking, aggregates formed cyst-like structures unlike any morphological arrangement previously reported. These consisted of three concentric layers, an outer non-cellular coat, a layer of loosely packed cells and a central core of tightly adhering cells (Fig. 3*a*). Both the outer coat and the surfaces of the outer layer of cells exhibited specific staining with antispore serum. Cells in the central core were not stained (Fig. 3*b*). On the basis of phase contrast microscopy we concluded that neither of the two cell types in the cysts structurally resembled either the spores or the stalk cells of normal fruiting bodies. Examination of stages intermediate between 24 and 96 h revealed a progressive accumulation of fluorescent-staining material at the aggregate surface and a corresponding decrease in the number and intensity of staining of PSVs. We suggest that although mature spores were not seen in these cysts, the vesicles in the prespore cells release their contents to the cell surface in the same way as do prespore cells during normal culmination[16,17], and that this material moves to the outside where it forms the coat of the cyst. Cyst formation is being further examined by electron microscopy. 4-d cysts did not develop when simply plated onto an agar surface but gave normal fruiting bodies within 4–5 d when plated with a bacterial food source.

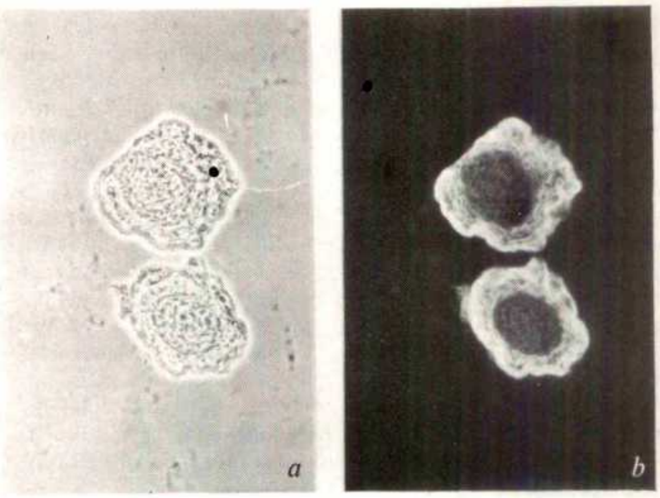
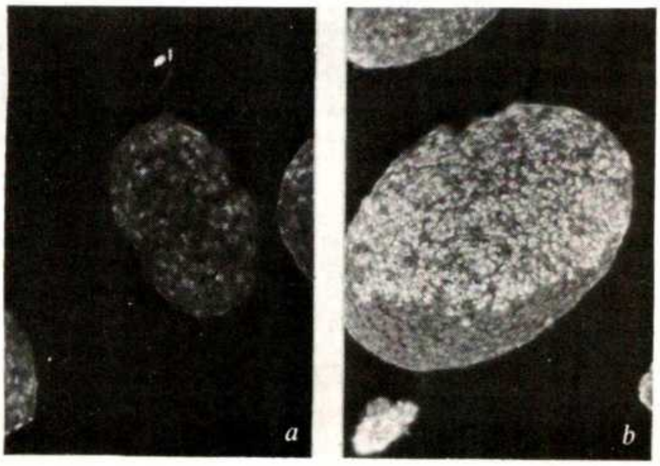


Fig. 3 Sections through 96-h aggregates under phase contrast (*a*) and ultraviolet light (*b*) having been stained with FITC-labelled anti-spore serum × 625.

Fig. 2 Sections through 18 h (*a*) and 24 h (*b*) aggregates stained with FITC-labelled anti-spore serum and observed under ultraviolet light. Aggregates were settled out from phosphate buffer, fixed in 95% ethanol, dehydrated, wax embedded, and sectioned at 5 μm according to the method of Sainte-Marie[30]. Staining was the same as for single cells. *a*, × 1,250; *b* × 625.



The most important conclusion from these results is that differentiation and pattern formation, ostensibly similar to that found in the normal life cycle, can occur amongst *D. discoideum* cells in conditions where anterior–posterior polarity and grex tip formation are absent. The only necessary conditions for prespore differentiation which we can identify at present are (1) that the cells should be 12 h post-feeding and (2) that they should be in mutual contact. PSV formation may be added to a number of differentiative processes mediated by cell contact following aggregation[18,19].

Since there is no obvious spatial organisation in the early appearance of prespore cells, we suggest, in common with Takeuchi[15], that the cell types differentiate at random within the aggregate and then give rise to the pattern by sorting-out. (That spatially random differentiation can occur within populations of slime mould cells is shown by the random establishment of aggregation centres within a uniform field of amoebae[20]. Proof that prespore and prestalk cells can also differentiate at random requires further experimentation.) Although our observations do not provide definitive proof of sorting-out, they are consistent with such an interpretation, and there is good evidence that sorting-out of slime mould cells does occur[7–11]. In addition it is well known from extensive studies on vertebrate embryo cells that different cell types adopt the types of configuration which we have found by sorting-out within aggregates[21,22]. Our results are inconsistent with differentiation in position because we have never observed in 18-h aggregates in a region devoid of prespore cells comparable in size to the non-staining regions of 24-h aggregates.

We propose the following new view of pattern formation in the slime mould. It has been shown that during the normal life cycle, differentiation of cell types slightly precedes the formation of a grex tip[23], and so it may be suggested that pattern formation occurs by sorting-out which begins before tip formation and that it is the early prestalk region which gives rise to the grex tip. Viewed in this way, it is the prestalk–prespore pattern which generates the polarity of the cell mass rather than the polarity which generates the pattern. Pattern formation certainly precedes the migrating grex stage[24] but it remains to be determined at precisely what stage during the normal life cycle the spatial pattern actually begins to form. Such a view leaves unexplained the mechanism governing the numerical proportions of mature spore and stalk cells which are known to be present in approximately the same proportionate ratio irrespective of the size of the cell mass[25–27]. We suggest (and we know of no evidence which contradicts this view) that the method of proportioning is independent of the mechanism which determines the spatial arrangement of cell types and the polarity of the cell mass.

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Defective chemotaxis in monosomy-7

CELLULAR defects of chemotaxis are rare disorders[1]. Little is known about the cellular mechanisms of the chemotactic response, and in most cases the nature of the defect remains obscure. Singh *et al.* have reported defective chemotaxis in association with a chromosomal abnormality[2]. In their patient, bone marrow cells were tetraploid. In most cells a chromosome 16 was missing and there was an extra C-group chromosome. We investigated neutrophil granulocyte function in haematological disorders. Our results from a study of neutrophil function in five patients with monosomy-7 seem to suggest an association between this chromosome abnormality and defective chemotaxis.

Patients 1 and 2 had blood and bone marrow findings indicating preleukaemia[3]; acute leukaemia later developed in patient 2. Patient 3 had acute myeloid leukaemia known to have been preceded by a preleukaemic phase. Patient 4 had acute myeloid leukaemia when first seen, and patient 5 had subacute myelomonocytic leukaemia. Patients 1 and 3 had distinctly increased susceptibility to bacterial infections, mainly pneumonias and abscesses. Patient 2 also suffered from repeated febrile episodes probably of infectious origin, but no conclusive evidence of infections could be obtained. Since defects of leukotactic function occur in chronic and acute bacterial infections[4,5], our tests were performed at least 1 week after the disappearance of any sign of infection. Patient 4 had been treated with cytostatic agents before testing, but not within 10 d before the experiments.

Chemotaxis was tested in patients 1, 2, 3 and 4 by a modification of the Boyden method[6] and in patient 5 by another modification[7] of the same. In the former method the results are expressed as the average number of cells per high power field that have migrated through a Millipore filter in 3 h, in the latter as the distance which the leading front of cells has migrated within the membrane in 1 h.

Table 1 Chemotaxis of neutrophils

Patient	Age (yr)	Sex	Cells	Chemotactic attractant	Cells per field
1	49	M	NC	NS 56° 30 min	0.2±0.2*
			NC	NS zy	65.0±3.0
			PC	NS zy	16.0±1.3
			NC	PS zy	112.7±4.9
2	49	M	NC	NS 56° 30 min	0.9±0.5
			NC	NS zy	71.2±4.2
			PC	NS zy	6.1±0.5
			NC	PS zy	87.4±4.8
3	29	F	NC	NS 56° 30 min	3.0±0.6
			NC	NS zy	52.4±4.1
			PC	NS zy	8.7±1.4
			NC	PS zy	55.7±2.9
4	56	F	NC	NS 56° 30 min	0±0
			NC	NS zy	25.6±2.1
			PC	NS zy	10.4±1.0
			NC	PS zy	23.1±1.5
					Distance migrated (µm)
5	68	F	NC	Hanks	19.6±0.9†
			PC	Hanks	17.2±0.5
			NC	Casein	87.4±3.9
			PC	Casein	70.5±2.1
			NC	NS 56° 30 min	51.5±1.8
			NC	PS 56° 30 min	48.1±1.5
			NC	NS zy	94.7±2.1
			NC	PS zy	89.3±4.1

Assay conditions: patients 1, 2, 3 and 4, cell concentration was adjusted to 2×10^6 neutrophils ml^{-1}, and 10% pooled human serum was used in the cell suspension throughout. Modified Boyden chambers[6] were set up in triplicate. 30% heat-inactivated (30 min at 56 °C) or zymosan-activated (30 min at 37 °C) serum was used in the lower compartment, as indicated in the table. Hanks' solution was used as the medium. The cells were allowed to migrate for 3 h at 37 °C. Results are expressed as the average number of neutrophils per high power field (×400) on lower surface of Millipore filters (3-µm pore size) separating the upper compartment of the chamber from the lower one. Cells were counted in ten high power fields on each filter. Patient 5, cell concentration was adjusted to 1×10^6 neutrophils ml^{-1}. No serum was used in the cell suspension. 10% serum was added to the attractant compartment as indicated. The cells were allowed to migrate into the filters for 55 min towards casein (5 mg ml^{-1}) or serum, and for 90 min towards Hanks' solution, after which the leading front[7] of the cells was determined in five fields of each of six filters. The mean ± 2 s.e. of our normal material (40 individuals) for migration towards Hanks' solution is 20.7±3.7 µm, and towards casein 99±18 µm.

*Mean ± 1 s.e. of ten high power fields in each of three filters.

†Mean ± 1 s.e. of five determinations in each of six filters.

NC, Normal cells; PC, patient cells; NS, pooled normal serum; PS, patient serum; zy, zymosan.

Table 2 Bone marrow chromosome findings

Patient	Time	Chromosome counts ≦43	44	45	46	47	≧69	Total	Most prevalent karyotype
1	XII 1972	1	2	18	—	—	—	21	45, XY, −7
	IX 1973	—	—	1	—	—	—	1	
	XI 1973	—	1	11	—	—	4	16	
	I 1974	—	5	11	—	—	1	17	
	III 1974	—	—	5	1	—	1	7	
	IX 1974	—	2	8	—	—	1	11	
2	X 1973	—	—	5*	21†	—	4	30	46, XY
	IX 1974	—	—	21	—	1	2	24	45, XY, −7, del (20) (q11)
	IV 1975	—	—	12	—	—	—	12	
3	IX 1973	—	—	11	9†	—	1	21	45, XX, −7
	X 1973	1	—	12	6†	—	1	20	
	VIII 1974	—	—	15	2	—	—	17	
	IX 1974	2	1	10	1†	—	1	15	
	V 1975	—	—	9	2†	—	—	11	
4	VIII 1974	—	—	13	—	—	—	13	45, XX, −7 (Ph^1+)
	IX 1974	—	—	19	1	—	—	20	
5	III 1974	—	1	14	—	—	2	17	45, XX, −7
	II 1975	—	—	21	—	—	—	21	
	III 1975	—	—	10	—	—	—	10	

* Two cells were 45, XY,–C.
† Normal karyotype.
In all patients the karyotypes of leukoagglutinin-stimulated peripheral lymphocytes were normal.

Casein is used as a chemoattractant in the latter modification in addition to serum. The concentration of mature neutrophils (band and segmented) was constant in all experiments.

Our method for bone marrow chromosome work has been described in detail previously[8]. The routine method for banding practised in all cases was Giemsa staining of trypsin-treated slides. Q- and C-banding was performed in selected cases.

The response of cells from patients 1, 2, 3 and 4 to zymosan-activated serum was clearly inferior to that of control cells (Table 1). Good chemotactic activity could be developed with patient sera. These results indicate a cell-associated defect of chemotaxis. Owing to a switch to a new method which also allows the measurement of the random migration of cells, the results of patient 5 are not quantitatively comparable with the others. A possibly reduced chemotactic response was seen in this case, also. The random migration (Hank's solution in the attractant compartment) of the patient's cells was not clearly different from that of control cells. The patient's serum behaved like normal serum. The analysis made so far does not allow us to make conclusions about the true nature of the defect, which might be in the stimulation of migration by chemoattractants (chemokinesis), or in detecting the chemotactic gradient (chemotaxis).

Monosomy-7 is one of a number of non-random chromosome abnormalities occurring in disorders of the human bone marrow[9]. We have diagnosed five examples of this abnormality (Table 2). The occurrence of a cellular defect of chemotaxis, which itself is a very rare disorder, in at least four of the five can hardly be coincidental. The question arises whether the defect is due to the chromosome abnormality itself or whether it is caused by the disease. All the patients had overt leukaemia or preleukaemia. We have investigated about 30 other patients with similar diseases, many of whom had various chromosomal abnormalities[10], as well as about 40 patients with infection problems, and found a cellular defect of chemotaxis in only one additional case. This was a patient with preleukaemia and trisomy-8, but in another patient with preleukaemia and trisomy-8 chemotaxis was normal[10]. Thus the incidence of a chemotactic defect in monosomy-7 seems to be very high compared with other patients with similar diseases.

The present results suggest that monosomy-7 is intimately associated with defective response to chemoattractants. Although the nature of the association and the causal relationships are obscure, it seems reasonable to assume that chromosome-7 carries a gene or genes important for the chemotactic or chemokinetic response of neutrophils. This phenomenon may be similar to the loss of the Colton blood groups in monosomy-7 (ref. 11).

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Age-dependent surface marker on haemopoietic stem cells

HAEMOPOIETIC stem cells, intravenously injected into lethally irradiated mice, form colonies in their spleens (colony-forming units in spleen, CFUS)[1]. This is a clonal assay[2]; CFUS form the precursors not only of red cells, granulocytes and platelets[3] but also themselves (self-renewal)[4]. Rosendaal *et al.*[5] reported that five spaced doses of hydroxyurea yielded femoral CFUS with a self-renewal

Table 1 Cellular products of the graft of circulating CFUS from anaemic mice

	Cell dose of graft in ml of donor blood	Colonies per spleen ±s.e.m.	DNA (μg) per spleen ±s.e.m.	DNA (μg) per colony ±s.e.m.	% ^{59}Fe uptake in recipients' blood ±s.e.m. (ERA)	%^{59}Fe uptake per colony ±s.e.m.	CFUC per spleen ±s.e.m. $\times 10^{-3}$	CFUC per colony ±s.e.m. $\times 10^{-3}$	Secondary CFUS per original colony
Irradiation control	Nil	0.3±0.2	309.9±15.6	—	0.5±0.2	—	0.6±0.2	—	—
RAMB	0.4	29.3±2.4	629.9±52.0	11.0±2.1	4.6±0.6	0.14±0.02	36.3±8.5	1.2±0.3	4.6±1.5
NRS	0.2	38.0±2.9	910.0±36.2	15.9±1.6	10.5±1.0	0.27±0.03	97.3±9.7	2.6±0.3	20.1±2.0

Normal, 3-month-old female BALB/c mice were the donors of the circulating CFUS used in these experiments. To increase the number of circulating CFUS mice were rendered anaemic by three consecutive daily subcutaneous injections of phenylhydrazine (Ajax Chemicals, Sydney, 1 mg per g body weight in neutral normal saline) and bled by cardiac puncture on the fifth day[7]. CFUS in blood were partly separated from red cells by dilution (1:1 normal saline) and centrifugation (800*g*, 20 min, RT) over a layer of Isopaque–Ficoll (Nyegaard, Oslo and Pharmacia, Uppsala, specific gravity 1.073). The layer of mononuclear cells at the plasma–Isopaque junction was collected. There was no detectable loss of CFUS with this technique. CFUS were exposed to NRS or adsorbed (BALB/c liver, red cells and thymus) RAMB[8], then complement (fresh frozen guinea pig serum) and finally washed before intravenous injection into recipients. Twice as many RAMB-treated cells than NRS-treated cells were injected so that the number of spleen colonies in recipients was about equal. Recipients (male BALB/c mice) were irradiated before grafting (20 per group) (700 R, 230 kVp, HVL ʟ mm Cu, 146 R min^{-1}, TSD 50 cm, Maximar) and received intravenous ^{59}Fe as the citrate 8 d later (0.5 μCi per mouse, ferric citrate, specific activity 100 μCi per 9.7 μg Fe). Half of each group was killed 10 d after grafting, cardiac blood was taken for estimation of the erythrocyte repopulating ability (ERA)[11] and spleens were taken for DNA estimation and colony counts[12]. 12 d after grafting the remaining mice were bled, killed and their spleens were collected for assay of CFUC and CFUS. CFUC were grown in α medium (Flow)–0.3% agar (Difco, Bacto Agar) for 14 d[9]. A single cell suspension of each spleen (passed through a sterile stainless steel sieve and gauze) was seeded—1/30 of an irradiation control spleen and 1/58 of a RAMB or NRS spleen. Dishes containing 5,000 normal nucleated myeloid cells were stimulated with the same colony-stimulating factor in the same culture and formed 100 colonies per dish. Secondary CFUS per original colony were assayed in pooled groups (irradiation control, RAMB and NRS, 10 mice per group).

capacity three times greater than that of untreated mice. A generation-age hypothesis was proposed as an explanation: the way the population of CFUS in the animal is organised to form blood is based on the generation-age of each CFUS after its origin in the animal. Younger stem cells have undergone fewer generation-ages so their capacity for self-renewal is greater. As stem cells age, the chances of their progeny being CFUS diminishes and the likelihood of their being cells committed to form red cells, granulocytes or platelets increases. It should be possible to demonstrate differences between stem cells with a reduced capacity to form themselves and those whose capacity is greater. I report here experiments with CFUS from the blood of anaemic mice treated with phenylhydrazine (PHZB CFUS)[6], whose capacity for self-renewal is known to be reduced[7].

PHZB CFUS were exposed to rabbit-anti-mouse-brain serum (RAMB, anti-CFUS serum)[8], or control normal rabbit serum (NRS) and then complement before grafting. The average NRS-treated PHZB CFUS formed four times more CFUS and twice as many colony forming units in culture (CFUC, committed precursors of granulocytes, progeny of CFUS)[9] and red cells than the average PHZB CFUS which had survived exposure to RAMB (Table 1). If allowance is made for the fact that 60% of the original PHZB CFUS had the RAMB determinant the differences were greater, threefold (CFUC and RBC) and sixfold (CFUS).

It is interesting that this same batch of RAMB killed 75% of normal bone marrow CFUS but only 50–60% (in different experiments) of PHZB CFUS. Furthermore, this RAMB had no effect on CFUC, progeny of CFUS, exposed to it before plating.

These results indicate that there are differences in the expression of the RAMB determinant between circulating stem cells with different capacities for self-renewal.

If the generation-age of stem cells were one of the ways in which blood formation were organised, it is appropriate to consider how that might be ordered in the animal. A distinction would have to be made between older and younger stem cells—an age-dependent surface marker on them might be one such means. Thus, as the stem cell aged, the expression of a receptor on it could diminish. Till has pointed out[10] that RAMB, which is expressed on some stem cells but not on CFUC, is a candidate surface marker. These results are compatible with his proposal. Experiments are in progress to elucidate the function of the RAMB determinant in the animal.

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Serum and hormonal regulation of the "resting-proliferative" transition in a variant of 3T3 mouse cells

3T3 CELLS are embryonic mouse fibroblasts established in culture[1]. Serum depletion or "step-down" causes them to enter a "resting" state, which they leave on restoration of serum or "step-up", entering the "proliferative" state[2–4]. This transition between resting (also called G$_0$, A or R) and proliferating states seems to be the critical step in cell growth control[5–7]. Its study has been aided recently by several observations on extracellular growth regulators for 3T3 cells, for example the discovery of a new pituitary growth factor[4,8], growth stimulation by glucocorticoids[4,9] and interactions between pituitary factor and classical hormones[10–12]. Further studies would be greatly helped if variants or mutants of 3T3 with different growth responses towards hormones could be isolated. Here we report our finding of the variant ST1 which is inhibited by glucocorticoids, unlike 3T3 cells whose growth is stimulated by glucocorticoids[4,9] (growth of fibroblasts is normally inhibited by glucocorticoids[13]). In this paper we discuss the value of this new cell type for studies of the resting–proliferative transition; elsewhere we shall report how the

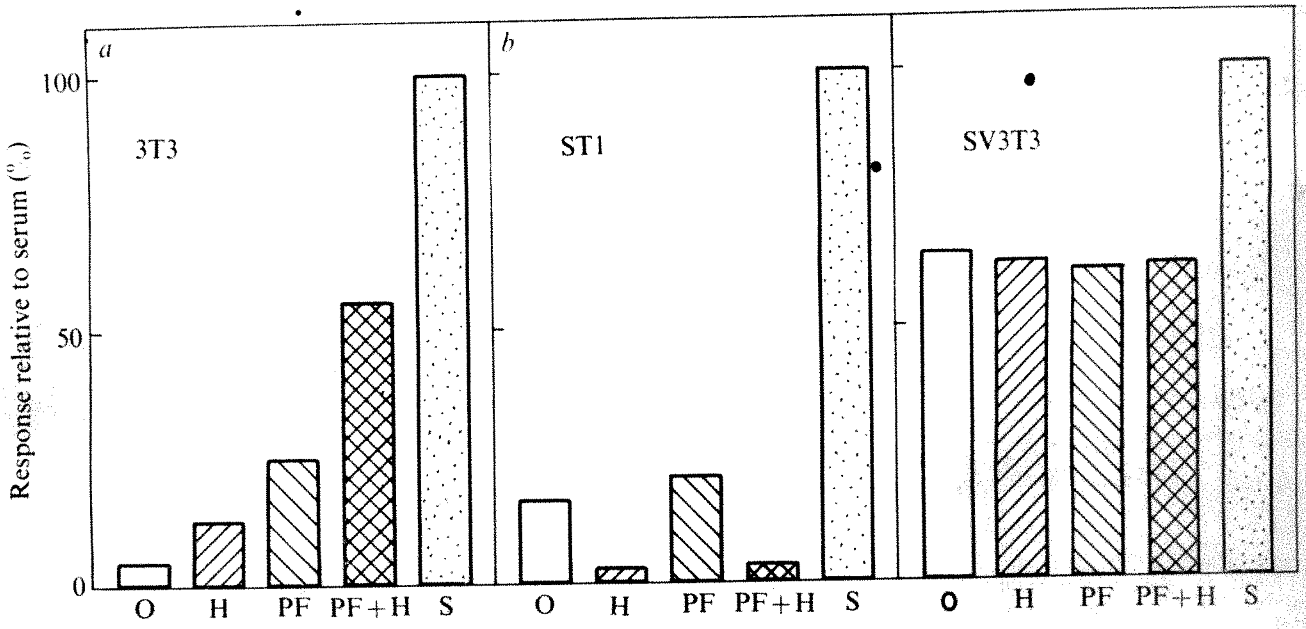
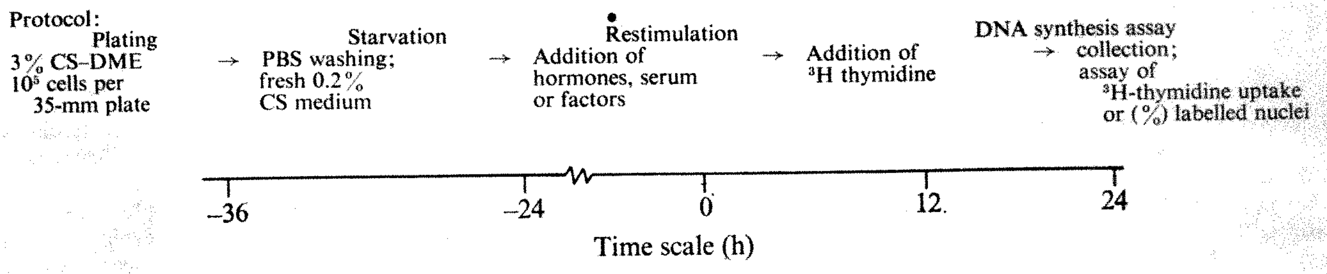


Fig. 1 Effect of hydrocortisone (H), pituitary factor (PF) and calf serum (S) on ^{3}H-thymidine uptake (0.25 μC ml^{-1}, 10^{-7} M) into DNA for three different clones of Swiss 3T3 cells (American Type Culture Collection).



▲ Abscissa shows additions to 0.2% calf serum at $t=0$. Two plates per point. Deviation was usually 5% and never greater than 10%. ^{3}H-thymidine uptake into DNA was as described in ref. 4. 100% corresponds to 3.3×10^4 c.p.m. per plate for clone 1; 4.9×10^4 c.p.m. per plate for ST1 and 5.1×10^4 c.p.m. per plate for SV3T3. PF is a growth factor preparation from frozen bovine pituitary glands containing seven bands in polyacrylamide gel electrophoresis (ref. 19).

adhesion of this variant to the substrate is greatly affected by both the removal of serum and the addition of cyclic AMP.

We sought new cell types by cloning mutagenised and non-mutagenised 3T3 populations; routinely confluent monolayers cultures were inspected for foci of piled-up variants. Other selection procedures have also been developed which will be reported elsewhere. In one particular case, repeated plating in low serum of non-mutagenised culture containing foci yielded a population enriched with variant cells which was then cloned and recloned. Since the variant clones obtained were all identical, one was chosen and called ST1 and the others were discarded.

Figure 1 represents the typical response of clones isolated from the parental 3T3 line, the ST1 variant and SV40-transformed 3T3 (SV3T3).

As can be seen from Table 1 and Fig. 1*b*, ST1 cells have: (1) diminished density inhibition of growth—they pile up and grow in methocel; (2) serum dependence; (3) half as many chromosomes as the parental line. Also their synthesis of DNA is not dependent on pituitary factor but is inhibited by hydrocortisone. In addition, insulin (at physiological or high doses) and a somatomedin-enriched serum factor have no effect on DNA synthesis but factor(s) contaminating commercial crystalline bovine serum albumin cause stimulation (results not shown).

The hydrocortisone inhibition of growth has the following very interesting features: (1) it is specific for glucocorticoids (sex steroids have no effect); (2) glucocorticoids are effective at physiological doses; maximal effect is obtained with 20 ng ml^{-1} dexamethasone or 50 ng ml^{-1} hydrocortisone; (3) the glucocorticoid growth inhibition depends on serum

Table 1 Properties of 3T3, ST1 and SV 3T3 cells

Cell line	Saturation density (cells cm^{-2})		Doubling time (h)		Growth in methocel	No. of chromosomes per cell
	10% CS	1% CS	10% CS	1% CS		
3T3	0.4×10^5	0.9×10^4	18	90	—*	80±2.5
ST1	4.1×10^5	2.5×10^4	14.5	38	+†	40±1
SV 3T3	4.5×10^5	$2\text{-}3\times10^4$	13	13	+	75.6±4.8

Saturation density and doubling time were determined using the same batch of calf serum. Cells were plated in methocel according to ref. 20. Karyotype analysis was done according to ref. 21. At least 10 metaphase plates were counted per cell line; figures are the mean ± s.e.m.

*No visible colonies after one month of culture; eventually presence of microcolonies of up to eight cells; plating density from 10^6 to 10^3 cells per plate; efficiency of plating: zero.

†Visible (2–4 mm) colonies counted 21 d after plating; efficiency of plating in different experiments: 1 to 3%.

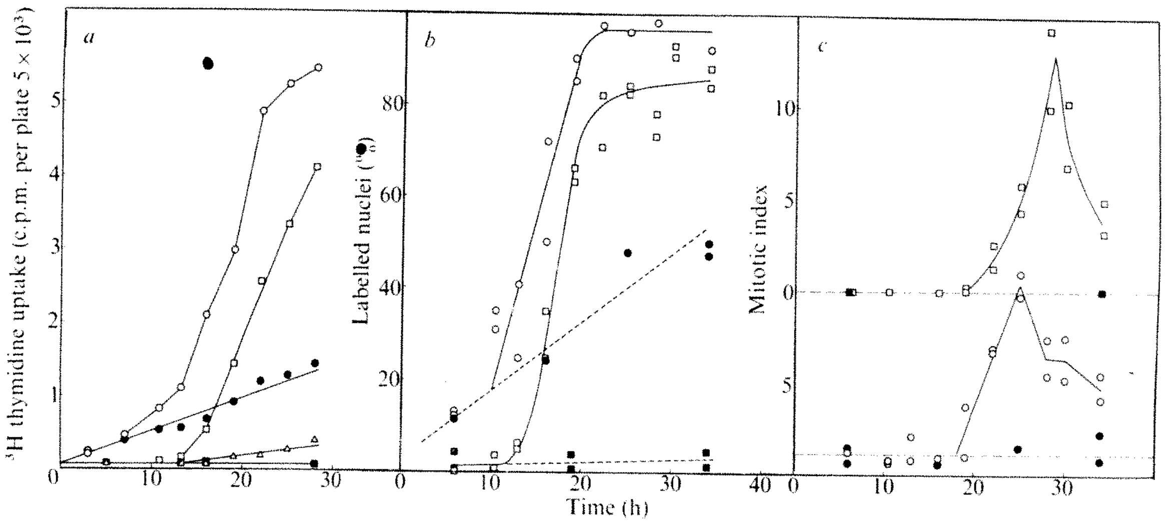


Fig. 2 Kinetics of restimulation with serum for ST1 cells treated with glucocorticoid and untreated. Cells were plated in 3% calf serum; 24 h later the medium was changed to 0.2% calf serum plus or minus hydrocortisone (300 ng ml^{-1}). After 24 h incubation in low serum, calf serum (5%) was added to restimulate cells. Plates treated with hydrocortisone were washed with PBS and received fresh 0.2% calf serum medium before serum addition. ^{3}H-thymidine (0.25 μCi ml^{-1}, 5×10^{-7} M) was added to all plates at the same time as serum. Plates were harvested at different time intervals after serum addition and assayed for: *a*, ^{3}H-thymidine uptake into DNA; *b*, percentage of labelled nuclei; *c*, mitotic index. ○, Plus serum, not treated with hydrocortisone; ●, no serum added, not treated; □, treated with hydrocortisone, steroid washed away, plus serum; ■, treated with hydrocortisone, steroid washed away, no serum added; △, treated with hydrocortisone, no serum added, steroid not washed away. Percentage of labelled nuclei determined by autoradiography using Kodak AR10 stripping film. Mitotic index determined in fixed and stained plates. Two plates per point for *a* (deviation never greater than 10%) one plate per point for *b* and *c* (at least 500 cells counted per plate in *b* and at least 1,000 cells per plate in *c*).

concentration: at 0.2% serum inhibition is 100% whereas at 5% serum it is 40%; (4) inhibition is completely reversible and glucocorticoid has no effect on cell viability: plating efficiency is the same (50–55%) in the presence or absence of hydrocortisone; (5) the inhibition, measured by 1-h ^{3}H-thymidine pulses, increases progressively with time after glucocorticoid addition, attaining its maximal level after 15–18 h of glucocorticoid addition; (6) glucocorticoids had no detectable effect on ongoing DNA synthesis or the ability of cells to undergo mitosis (data not shown); (7) 100% of the cells from an exponentially growing culture are found in a "resting" state 15–20 h after serum step-down in the presence of hydrocortisone; these "resting" cells are fully responsive on serum restimulation (Fig. 2).

The results of kinetic experiments, shown in Fig. 2, in which ^{3}H-thymidine labelling and mitotic index were followed, indicate that: (1) serum step-down causes accumulation of cells at the beginning of G_1 or at G_0, though in the absence of hydrocortisone residual DNA synthesis and growth remain; (2) from the kinetics of the stimulated DNA synthesis one can make estimates of the length of G_1 (Table 2): in the presence of hydrocortisone G_1 was longer by 4.0 h (^{3}H-thymidine uptake) or 3.8 h (labelled nuclei) than in its absence; (3) the increment in mitotic index was delayed by 3.8 h (Table 2) in cells treated with hydrocortisone, indicating that hormone-treated and untreated cells traverse S and G_2 at the same rate.

In conclusion, it seems clear to us that glucocorticoids at physiological doses inhibit growth of ST1 cells without compromising their viability or interfering with biochemical processes of S, G_2 or mitotic phase of the life cycle. This inhibitory effect seems to be directly connected with the mechanism which regulates the transition between "resting" and "proliferative" states of the cell.

Resting "normal" 3T3 cells when restimulated by serum step-up or PF addition enter the S phase, after identical G_1. Addition of hydrocortisone to PF further increases the stimulation, accelerating the entry of cells into S, but without interfering with G_1 length (M.C.S.A. and H.A.A., unpublished). This accelerating effect is due to an increase in the probability of cells exiting in the resting state. On the other hand the glucocorticoid seems to cause a resetting of the growth control mechanisms of ST1 cells, leading to a strict "resting" state with serum step-down and a larger G_1 upon restimulation by serum step-up. We feel that our approach to the growth regulation of 3T3 cells and its variants might yield new information about the mechanisms of cell growth control.

It has long been recognised that glucocorticoids inhibit the growth of fibroblasts *in vivo* and *in vitro*[13], but the mechanisms of this effect are still unknown. In culture this glucocorticoid-inhibiting effect has been studied using L cells, a line of tumorigenic mouse fibroblasts[14–17] and has also been observed with normal chick embryo cells[18]. It

Table 2 Estimates of G_1 and T (G_1+S+G_2) on restimulation of ST1 cells with serum

Kinetics of	G_1^{+H}	G_1^{-H}	(G_1^{+H}—G_1^{-H})	T^{+H}	T^{-H}	(T^{+H}—T^{-H})
^{3}H-thymidine uptake into DNA	1) 14.5 2) 14.5	1) 10.4 2) 10.6	1) 4.1 2) 3.9	—	—	—
Nucleus labelling	2) 13.9	2) 10.1	2) 3.8	—	—	—
Mitotic index	—	—	—	2) 21.8	2) 18.0	2) 3.8

G_1^{+H} and G_1^{-H} are the length of G_1 (in hours) for cells treated with hydrocortisone and untreated, respectively. T^{+H} and T^{-H} are the period of time equivalent to G_1+S+G_2 for treated and untreated cells, respectively. G_1 was estimated by taking the best-fitting straight lines from kinetic curves like the ones shown in Fig. 2*a* and *b* in the interval 0 to 22 h for non-stimulated and 11 and 22 h for stimulated cultures. The abscissa corresponding to the intercept of these two lines was taken as G_1 (or the length of time a cell takes to leave the resting state and initiate DNA synthesis or enter the S phase, see refs 5, 6 and 7) Estimates of T were obtained in a similar manner using mitotic index curves like the ones shown in Fig. 2*c*. 1 and 2 correspond to estimates obtained from two completely independent experiments.

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would be interesting to find out whether the pattern of inhibition we describe for ST1 cells is a common response for fibroblasts. Such a suggestion is not unreasonable considering the results reported for chick cells[18] and L cells[17].

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Interaction of two hormones and their effect on observed rate of initiation of DNA synthesis in 3T3 cells

THE rate of replication of mammalian cells changes in response to alterations in the external environment usually by expansion or contraction of the average time of the G_1 phase of the cell cycle; the remaining phases are relatively constant (S, G_2, M)[1–3]. Thus, when varying concentrations of serum[4] or the growth-promoting[5–7] factors are added to relatively quiescent fibroblastic cells variations in rates of cell division are mainly due to differences in rates of initiation of DNA synthesis[4,6]. Recently Brooks[8] has shown that addition of serum and adenosine to quiescent BHK fibroblasts stimulated the cells to enter S phase with first-order kinetics, although without adenosine the kinetics were more complicated. We now report that the stimulation of DNA synthesis induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can be described as an increase in a first-order rate constant for exit from the G_1 phase preceded by a lag phase of relatively fixed duration. Insulin and certain nutrients which increase the effect of $PGF_{2\alpha}$ upon DNA synthesis do so by changing the same rate constant.

Swiss mouse 3T3 fibroblastic cells[9] were allowed to grow and become quiescent either in Dulbecco's modified Eagle's medium (DEM) and 6% serum or in the same supplemented with additional nutrients (DMS) (Fig. 1). Addition of increasing concentrations of $PGF_{2\alpha}$ (0–200 ng ml^{-1}) to the cultures caused an increasing percentage of cells to synthesise DNA in 30 h (labelling index) up to a maximum, at 200 ng ml^{-1} of 12% or 23% in DEM or DMS respectively. Addition of a physiological concentration (50 ng ml^{-1}) of insulin alone induced only 4% or 5% of the 3T3 cells in DEM or DMS to synthesise DNA. However, addition of insulin markedly increased the response to all concentrations of $PGF_{2\alpha}$ in either medium up to a maximum labelling index of 24% and 43% in DEM or DMS respectively (synergistic effect) (Fig. 1).

Two concentrations of $PGF_{2\alpha}$ (30 and 300 ng ml^{-1}) with or without insulin were added to quiescent cultures and the cumulative labelling index was subsequently followed with time (Fig. 2). The same results were plotted as the logarithm of the percentage of unlabelled cells against time[8]. Since most of the quiescent cells (95%) were in G_1[10] the gradient of the curves in Fig. 3 gave the first order rate constant at which G_1 cells were entering the S phase at a given time. Few cells became radioactively labelled in the first 15–16 h (lag phase). The duration of the lag phase was independent of the concentration of $PGF_{2\alpha}$ with or without insulin in DEM or DMS, and in accordance with previous results for varying serum[4] or FGF[6] concentrations. At the end of the lag phase however, the rate at which cells entered S increased abruptly and within 2 h the rate constant reached a steady value (Fig. 3). Straight lines

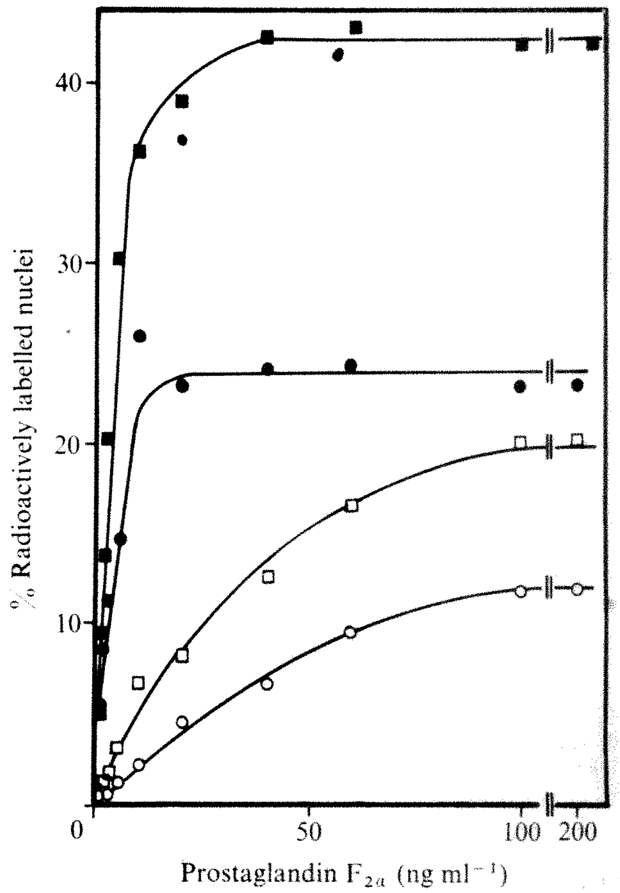
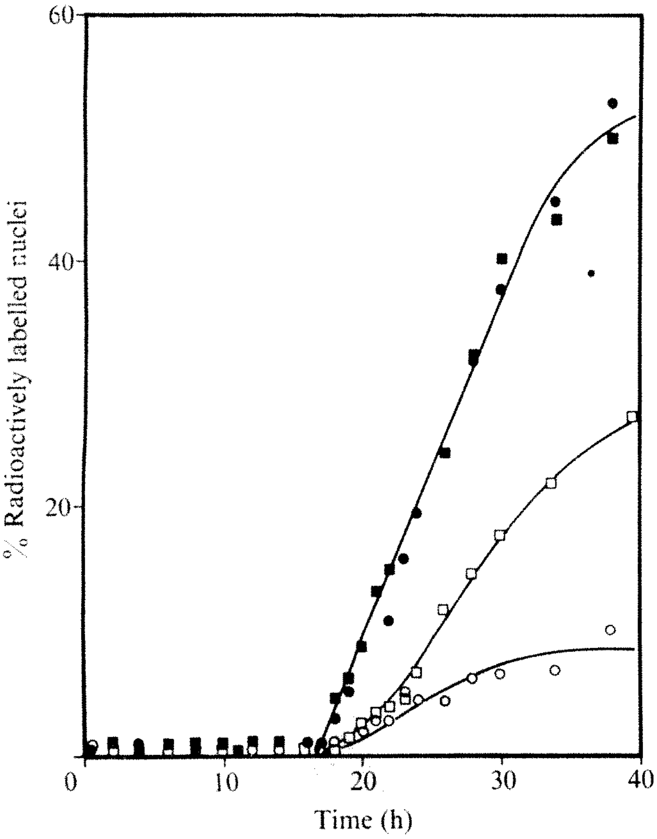


Fig. 1 Effect of different concentrations of $PGF_{2\alpha}$ on the percentage of cells synthesising DNA in 30 h. Increasing concentrations of $PGF_{2\alpha}$ were added to quiescent 3T3 cells in Dulbecco's modified Eagles medium (DEM) without (○—○) and with (●—●) 50 ng ml^{-1} insulin or to quiescent cells in DEM supplement with low molecular weight nutrients (DMS) without (□—□) and with (■—■) insulin. Swiss mouse 3T3-4 fibroblasts[9] were maintained in DEM containing 100 units ml^{-1} penicillin, 100 ng ml^{-1} streptomycin and 10% foetal calf serum. Subconfluent cultures were grown in 90 mm Nunc Petri dishes at 37 °C under a pressure of 10% CO_2 and were routinely checked for any contamination by mycoplasma[7]. Cells were plated at 10^5 per 33 mm dish in 2 ml of DEM and 6% foetal calf serum, or in DEM and 6% serum supplemented with 0.03 μM biotin, 0.05 μM vitamin B_{12}, 140 μM aspartic acid, 0.3 mM glutamic acid, 50 μM glutathione, 60 μM hypoxanthine. All additional nutrients were required to obtain the maximum stimulation of DNA synthesis by $PGF_{2\alpha}$ and insulin in 3T3 cells. All additions were made at the same time and then 3 μCi ml^{-1} methyl-^{3}H-thymidine was added at 1 μM and the cells were left for 30 h. Cultures were then processed for autoradiography as previously described[6,7], and the percentage of cells with radioactively labelled nuclei was recorded. In parallel experiments cells were radioactively labelled with 3 μCi ml^{-1} ^{3}H-thymidine at 3 μM for the same time and the radioactivity incorporated into DNA[6,7] was determined for no additions (1,500 c.p.m.); 200 ng ml^{-1} $PGF_{2\alpha}$ (26,500 c.p.m.); $PGF_{2\alpha}$ and 50 ng ml^{-1} insulin (87,300 c.p.m.) in DEM; or no additions (1,800 c.p.m.); $PGF_{2\alpha}$ (70,000 c.p.m.); $PGF_{2\alpha}$ and insulin (144,000 c.p.m.) in DMS.

fitted the data well indicating an underlying first-order reaction or event defined by an apparent rate constant k. The goodness of fit of regression lines to the data of Fig. 3*a* and *b* was estimated by calculating coefficients of determination r^2. These ranged from 0.93 to 0.98 where $r^2 = 1$ implies a perfect fit. PGF$_{2\alpha}$ at a concentration of 30 ng ml^{-1} in DMS gave $k = 6.0 \pm 0.5 \times 10^{-3}$ h^{-1} while at 300 ng ml^{-1} $k = 1.5 \pm 0.1 \times 10^{-2}$ h^{-1}. Insulin with either 30 or 300 ng ml^{-1} of PGF$_{2\alpha}$ dramatically increased k to a value of $3.5 \pm 0.2 \times 10^{-2}$ h^{-1}. When 300 ng ml^{-1} PGF$_{2\alpha}$ and insulin were added to cells which had become quiescent in DEM or DMS the value of k was $2.2 \pm 0.1 \times 10^{-2}$ h^{-1} in DEM and $5.1 \pm 0.3 \times 10^{-2}$ h^{-1} in DMS. The corresponding value for 10% serum in DMS was approximately four times as high ($k = 0.24 \pm 0.05$ h^{-1}). Variations in absolute k values for additions of these hormones were sometimes observed for different batches of cells and serum, but the kinetics were always first order. When quiescent cells in DEM were radioactively labelled for up to 5 d (not shown) a slight but constant gradient was observed ($k = 6.4 \pm 0.5 \times 10^{-4}$ h^{-1}), presumably due to a slow rate of cell division (R. Shields and J. A. Smith, personal communication). Thus, there was probably a similar low rate of initiation of DNA synthesis during the lag phase (Fig. 3). Time-lapse cinematography of cultures indicated that cell death and detachment were occurring and this probably balanced the slow rate of cell division (R. Shields, unpublished results). When PGF$_{2\alpha}$ and insulin were not added together, but insulin was added 9 h later, the kinetics of entry into S were still first order and the rate constants were similar (Fig. 3*c*). However, when insulin was added at the end of the lag phase,

Fig. 2 Changes in the labelling index with time after addition of PGF$_{2\alpha}$ and insulin. The following additions were made: PGF$_{2\alpha}$, 30 ng ml^{-1} (○—○); PGF$_{2\alpha}$, 30 ng ml^{-1} + insulin, 50 ng ml^{-1} (●—●); PGF$_{2\alpha}$, 300 ng ml^{-1} (□—□); and PGF$_{2\alpha}$, 300 ng ml^{-1} + insulin, 50 ng ml^{-1} (■—■). The cells were grown in DMS and 6% foetal calf serum and allowed to become quiescent (Fig. 1). After additions the cultures were exposed to ^{3}H-thymidine from 0 until the indicated times. Pairs of cultures were then processed for autoradiography and the total fraction of cells synthesising DNA in that time period (labelling index) was recorded.



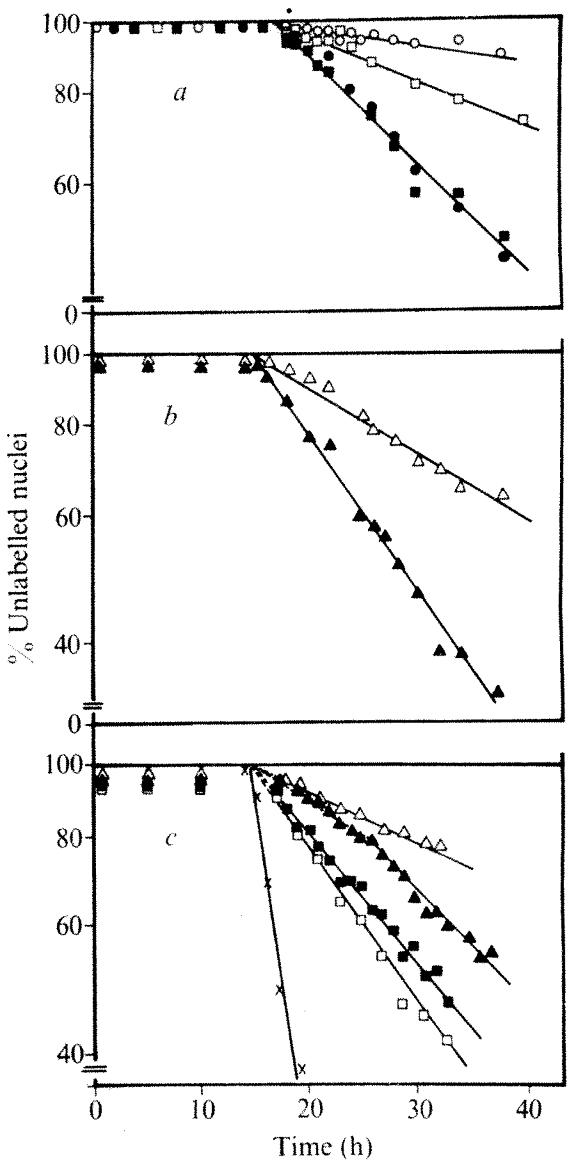


Fig. 3 *a*, Fraction of cells remaining in G$_1$ after synchronous addition of PGF$_{2\alpha}$ and insulin. The results in Fig. 2 were plotted as the logarithm (base 10) of the percentage of cells remaining in G$_1$ (percentage of non-labelled cells) $\log_{10}y$ against time (t) in hours (see below). The symbols were as follows: PGF$_{2\alpha}$, 30 ng ml^{-1} (○—○); PGF$_{2\alpha}$, 30 ng ml^{-1} + insulin, 50 ng ml^{-1} (●—●); PGF$_{2\alpha}$, 300 ng ml^{-1} (□—□); and PGF$_{2\alpha}$, 300 ng ml^{-1} + insulin, 50 ng ml^{-1} (■—■). Regression lines given by $\log_{10}y = a - bt$ were subsequently calculated for times greater than 15 h. The constants (a, b), with standard errors, were for PGF$_{2\alpha}$, 30 ng ml^{-1}; PGF$_{2\alpha}$, 30 ng ml^{-1} + insulin; PGF$_{2\alpha}$, 300 ng ml^{-1}; PGF$_{2\alpha}$ 300 ng ml^{-1} + insulin: (2.029 ± 0.004, 0.0026 ± 0.0002); (2.268 ± 0.017, 0.0154 ± 0.0007); (2.121 ± 0.013, 0.0066 ± 0.0005); (2.248 ± 0.017, 0.0148 ± 0.0007). Testing the goodness of fit yielded values of coefficients of determination (r^2) of 0.93, 0.98, 0.96 and 0.98 respectively. *b*, Fraction of cells remaining in G$_1$ in DEM and DMS after addition of PGF$_{2\alpha}$ and insulin. PGF$_{2\alpha}$, 300 ng ml^{-1} and insulin, 50 ng ml^{-1} were added to quiescent cultures grown in DEM (△—△) or DMS (▲—▲) and 6% serum. Cultures were processed as described in Fig. 2 and plotted as above. The constants (a, b), with standard errors, for the regression lines $\log_{10}y = a - bt$, were for DEM and DMS: (2.150 ± 0.015, 0.0096 ± 0.0006) and (2.332 ± 0.037, 0.0221 ± 0.0014) while $r^2 = 0.96$ and 0.97 respectively. *c*, Fraction of cells remaining in G$_1$ after non-synchronous additions of PGF$_{2\alpha}$ and insulin. PGF$_{2\alpha}$, 300 ng ml^{-1} was added to quiescent cultures grown in DMS and 6% serum. Insulin (50 ng ml^{-1}) was added at various times afterwards, as follows: no insulin (—△—△—); insulin at 17 h (—▲—▲—); insulin at 9 h (—■—■—); insulin at start with PGF$_{2\alpha}$, (—□—□—). Controls with fresh 10% serum added at the start, (—×—×—). Cultures were processed and the results plotted as above. The values for the first-order rate constants k for addition of PGF$_{2\alpha}$ with no insulin; with insulin after 17 h; with insulin after 9 h; and with insulin at the start were: 1.6 ± 0.3; 3.3 ± 0.7; 3.9 ± 0.5; and $4.7 \pm 0.8 \times 10^{-2}$ h^{-1} respectively. For 10% serum from the start $k = 0.24 \pm 0.05$ h^{-1}.

Table 1 Stimulation of rate of 2-deoxyglucose uptake, rate of cellular protein synthesis and increase in rate constant (*k*)

	2-deoxyglucose* (%)		Protein synthesis† (%) 5 h		Rate constant (*k*) for entry into S‡ (%)
Additions	1 h	5 h	Methionine	All amino acids	
None	100	100	100±4	100±9	100
Insulin	312	372	124±8	107±6	310
$PGF_{2\alpha}$	110	565	127±3	120±4	740
$PGF_{2\alpha}$+insulin	340	1,890	206±11	198±4	1,740
Serum	350	3,758	228±8	210±4	—

3T3 cultures were grown and allowed to become quiescent in DEM as in Fig. 1. Then 300 ng ml^{-1} of $PGF_{2\alpha}$, 50 ng ml^{-1} insulin, 10% foetal calf serum were added as indicated. After 45 min or 4 h 45 min, 2-deoxyglucose uptake was measured[19]. The cell monolayers were washed three times with prewarmed serum-free medium without glucose and then incubated for 15 min in the medium with hormones or serum. The cells were then exposed to 3 μCi ml^{-1} of 2-deoxyglucose at 50 μM for 15 min. Uptake into the fraction soluble in 5% trichloroacetic acid was linear for this time[19].

*Results expressed relative to the incorporation for no additions (320 c.p.m. per min per mg protein). Protein synthetic rates were determined by exposing the cultures to either 10 μCi ml^{-1} ^{35}S-methionine or 0.5 μCi ml^{-1} ^{14}C total amino acids in the normal medium from 2 h until 5 h after the additions and then removing samples every 30 min up to 6 h after adding the isotope. Cell monolayers were washed twice with isotonic saline, dissolved in 2.0 ml of 0.5 M NaOH and divided into two equal fractions. Total protein content was determined in one fraction[20], while the second fraction was incubated 1 h at 37 °C to hydrolyse the aminoacyl tRNAs. This was then precipitated with trichloroacetic acid and analysed for radioactivity[21]. The incorporation of radioactivity into the TCA-soluble pool was constant at 3 and 6 h and independent of hormonal additions, and the incorporation of radioactivity into protein per mg cell protein was linear with time for the 6-h interval[21].

†Results expressed as a percentage of the value for no additions (^{35}S-methionine, 100% = 53,500 c.p.m. per h per mg protein; ^{14}C total amino acids 100% = 7,300 c.p.m. per h per mg protein). Results ± standard errors are the means of the rates of incorporation measured over 6 h in duplicate experiments.

‡The rate constant (*k*) for cellular entry into S was calculated from the radioactive labelling index at 30 h assuming *k* to be constant and is expressed as a percentage of the value for no additions (100% = 0.10% of cells entering S h^{-1}. No value of *k* was calculated for serum addition since the rate of entry into S does not follow first-order kinetics under these conditions[8], but the labelling index at 30 h was 88%.

the rate constant gradually increased. After 9 or 10 h the rate constant was nearly the same as that for simultaneous additions. This suggested that when insulin was added near the completion of the $PGF_{2\alpha}$-induced lag phase changes in the rate constant for entry into S could be produced without appreciable delay.

$PGF_{2\alpha}$ and insulin separately increased protein synthetic rates about 1.2-fold, but when added together they increased the rates by a maximum of about 2-fold after 5 h (Table 1). Synergistic effects of $PGF_{2\alpha}$ and insulin were observed in the protein synthesis-dependent increase in 2-deoxyglucose uptake (Table 1) which also started after 2–6 h. The latter increases were much larger (19-fold) than those in total protein synthesis and closely reflected the increased rate constants for entry into S (ref. 12) (Table 1). No synergistic effects, however, were observed in the initial protein synthesis-independent increases in 2-deoxyglucose (Table 1) rubidium[13] or phosphate[13] uptakes.

Our results are compatible with the two models of the cell cycle proposed by Smith and Martin[14] and by Pardee[15]. In the former model cells enter an indeterminate state (A state) in G_1. From this they proceed to the determinate phase (B phase) of the cell cycle (S, G_2, M and remainder of G_1) at random with a constant probability (*P*). This predicts first-order kinetics of initiation of DNA synthesis in constant conditions. The growth stimulation of quiescent cells is then a switching of the cell population from one A state to another of higher *P*. In the second model the major growth regulatory point in a cell exists as a single restriction point (R point) in G_1 for critical nutrients and growth-stimulatory agents. Readdition of these components to deprived cells results in a constant lag phase prior to DNA synthesis.

A single, rate-limiting step or event is strongly suggested by the first-order kinetics of cellular entry into S phase. This step may occur close to the onset of DNA synthesis[16]. As shown above, $PGF_{2\alpha}$ and insulin interact synergistically upon the rate of the cells' entry into the S phase, and thus presumably upon that step. Interaction also occurs in some of their effects involving protein synthesis although not always at their first presumed site of action in the plasma membrane[17,18]. It is thus possible that the interaction upon DNA synthesis may, like that upon 2-deoxyglucose uptake, depend on synergy upon synthesis of protein(s). This is not inconsistent with the ability of insulin when added later than $PGF_{2\alpha}$ to affect the initiation of DNA synthesis within a few hours. As the duration of the lag phase appears independent of serum[4,6,8,11], or hormonal concentrations[6] the events determining this duration may be partly different from those by which hormonal concentrations determine the rate of initiation of DNA synthesis.

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Cyclic AMP regulation of mitogen-induced interferon production and mitogen suppression of immune response

In a recent review, adenosine 3′,5′-cyclic monophosphate (cyclic AMP) was proposed to have an inhibitory effect on the immunological and inflammatory functions of leukocytes[1]. Guanosine 3′,5′-cyclic monophosphate (cyclic GMP) has been shown to reverse the inhibitory effect of cyclic AMP in the *in vitro* immune response, and it has been proposed that the ratio of cyclic AMP to cylcic GMP determines whether an immune response will be enhanced or suppressed[2]. Cyclic AMP has recently been shown to inhibit the production of several soluble lymphocyte mediators (lymphokines) under antigen or mitogen stimulation[3–5]. T lymphocyte mitogens are potent inhibitors of the *in vitro* immune response as a result of stimulation of suppressor cell activity[6–8]. They are also potent stimulators of the synthesis of a unique type of interferon by T lymphocytes[9–11]. Evidence suggesting that this interferon may be the mediator of some forms of suppressor T cell activity has been presented[11]. I examine in this report the ability of exogenous cyclic AMP, and agents that increase the endogenous level of cyclic AMP, to block both the production of mitogen-type interferon and the development of the suppressor state in an *in vitro* murine antibody system. The data show that cyclic AMP blocks both aforementioned T cell functions, and are consistent with the hypothesis that interferon may be the mediator of some forms of suppressor cell activity[11].

Figure 1 presents data showing dibutyryl cyclic AMP inhibition of the production of interferon in C57B1/6 mouse spleen cell cultures that were stimulated by the two T cell mitogens concanavalin A (con A)[12] and staphylococcal enterotoxin A (SEA)[8]. Dibutyryl cyclic AMP, 2×10^{-5} M, inhibited con A stimulation of interferon production by 96%, while 1×10^{-4} M inhibited SEA stimulation of interferon production by 85%. When the same concentrations of dibutyryl cyclic AMP were added to the supernatant fluids of mouse spleen cell cultures after complete interferon production, no inhibition of antiviral activity of the interferon was observed, so it is concluded that dibutyryl cyclic AMP blocked the production of interferon, and not the established activity of produced interferon.

Table 1 presents data on dibutyryl cyclic AMP blockade of mitogen-induced suppression of the *in vitro* plaque-forming cell response to sheep red blood cells (SRBC). SEA

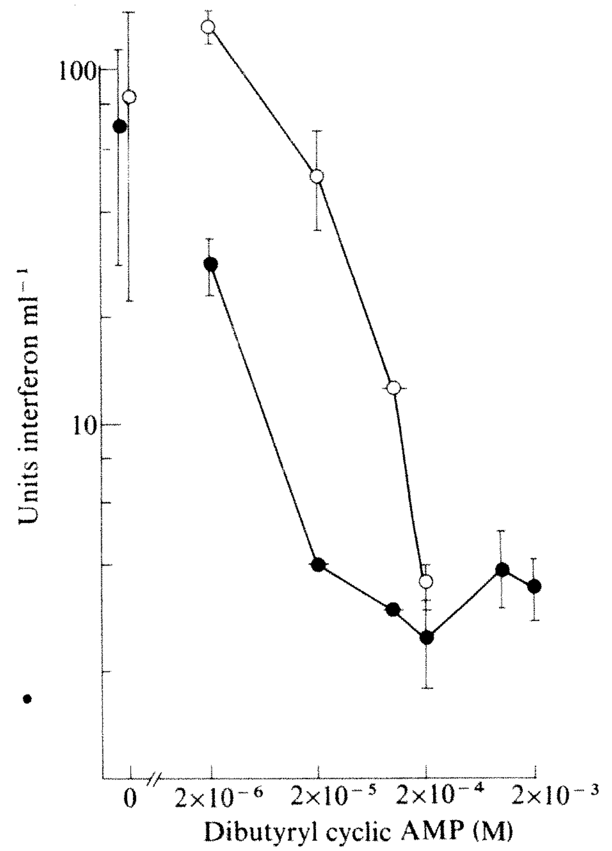

Fig. 1 Dibutyryl cyclic AMP inhibition of the production of interferon in concanavalin A (con A, ○) and staphylococcal enterotoxin A (SEA, ●) stimulated C57B1/6 female (8 week old) mouse spleen cell cultures. Cultures consisted of 1.5×10^7 spleen cells per ml and 2 μg and 1 μg con A and SEA, respectively. The con A and SEA are the same mitogens used previously[11]. Dibutyryl cyclic AMP was obtained from Calbiochem, San Diego, California. Dibutyryl cyclic AMP and mitogens were added to cultures in modified minimal essential media (MEM) (100-μl volumes) at the same time and the cells were incubated for 48 h as previously described[11]. Supernatant fluids were obtained by centrifugation of the collected cultures at 1,000 r.p.m. in a RC-3 Sorvall centrifuge at 7 °C. Fluids were assayed on the day of collection for mitogen-induced interferon activity in mouse L cells by a slight modification of the method of inhibition of cytopathic effect of vesicular stomatitis virus (100 $TCID_{50}$ per challenge dose)[13]. Data are plotted as units of interferon ml^{-1} ± s.d. for duplicate samples.

Table 1 Effect of dibutyryl cyclic AMP on mitogen-induced suppression of the *in vitro* PFC response to SRBC

Mitogen	Mitogen concentration (μg ml^{-1})	Dibutyryl cyclic AMP (1.4×10^{-4} M)	PFC per culture ± s.d.	PFC per 10^6 viable cells ± s.d.
SEA	0.25	—	350 ± 71	64 ± 8
SEA	0.50	—	550 ± 71	101 ± 11
SEA	0.25	+	13,150 ± 2051	5,080 ± 820
SEA	0.50	+	13,500 ± 1414	5,508 ± 1406
—	—	+	9,650 ± 2616	4,148 ± 296
—	—	—	18,350 ± 4596	5,206 ± 3626
Con A	1.0	—	25 ± 35	7 ± 9
Con A	2.0	—	100 ± 71	16 ± 11
Con A	1.0	+	1,900 ± 566	1,577 ± 505
Con A	2.0	+	1,750 ± 424	1,456 ± 307
—	—	+	9,775 ± 1308	3,146 ± 537
—	—	—	15,325 ± 5409	2,571 ± 691

Dissociated normal female (8 week old) C57B1/6 mouse spleen cells were cultured for *in vitro* anti-SRBC plaque-forming cell (PFC) response exactly as described by Mishell and Dutton[14]. Cultures consisted of 1 ml of 1.5×10^7 spleen cells ml^{-1} and 3×10^6 SRBC. Dibutyryl cyclic AMP was added to cultures 1 h before mitogen and SRBC addition. Dibutyryl cyclic AMP and mitogens were diluted in modified MEM, and added to cultures in 100 μl volumes. All PFC responses were determined at day 5. Cell viabilities were determined by trypan-blue dye exclusion. Direct PFC assays were carried out on microscope slides as previously described[15]. Results are expressed as the average of duplicate cultures ± s.d.

Table 2 Effect of agents that increase endogenous cyclic AMP levels on SEA-induced suppression of the *in vitro* PFC response to SRBC

Agent (μg ml^{-1})	SEA (0.5 μg ml^{-1})	PFC per culture ±s.d.	PFC per 10^6 viable cells ±s.d.
—	+	1,625±247	431±135
Cholera toxin (0.1)	+	9,600±1061	2,984±596
Cholera toxin (0.1)	—	7,225±1096	4,791±165
—	—	13,150	4,326
—	+	500±71	91±42
IMX (12.5)	+	5,300±424	3,973±477
IMX (12.5)	—	3,500±1556	1,366±571
—	—	9,850±283	2,294±1238

Cholera toxin and IMX (3-isobutyl-1-methyl xanthine) were added to *in vitro* plaque-forming cell (PFC) cultures under the same conditions as described in Table 1. IMX was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Results are expressed as the average of duplicate cultures ±s.d., except where only one culture was assayed.

at 0.25 and 0.50 μg ml^{-1} inhibited the plaque-forming cell response by >90% when compared to cultures receiving either SRBC alone or dibutyryl cyclic AMP along with the SRBC. Protection of the plaque-forming cell response was essentially complete when dibutyryl cyclic AMP (1.4×10^{-4} M) was added to cultures along with SEA. The protection of the anti-SRBC plaque-forming cell response was significant when expressed either as plaque-forming cells per culture or plaque-forming cells per 10^6 viable cells. Dibutyryl cyclic AMP protection of the plaque-forming cell responses from con A suppression was also observed. Con A at 1.0 and 2.0 μg ml^{-1} inhibited the plaque-forming cell response by >90% when compared to the controls. Blockade of suppression was more dramatic when the plaque-forming cell response was expressed in terms of viable cells recovered, since the presence of con A and dibutyryl cyclic AMP in the same culture resulted in relatively fewer viable cells recovered as compared to the presence of the two agents in separate cultures. The control containing dibutyryl cyclic AMP and SRBC produced fewer plaque-forming cells per culture than that containing only SRBC, but the responses were essentially the same when expressed as plaque-forming cells per 10^6 viable cells. The reduction in the plaque-forming cell responses due to the dibutyryl cyclic AMP alone, then, can be attributed to the smaller number of viable cells recovered after incubation. This latter observation is in agreement with a recent report[16] that showed that cyclic AMP did not inhibit the *in vitro* immune response at concentrations used here. Repeated experiments showed the same pattern of dibutyryl cyclic AMP blockade of mitogen-induced suppressor activity. Further, dose–response studies with various concentrations of dibutyryl cyclic AMP showed that the concentrations (1×10^{-4} to 2×10^{-4} M) of the cyclic ribonucleotide that blocked the development of suppressor activity correlated with those concentrations that blocked the production of interferon in spleen cultures (Fig. 1) stimulated by the T-cell mitogens. Dibutyryl cyclic GMP, at the same concentrations, had no effect on either mitogen stimulation of interferon production or mitogen-induced suppression of the *in vitro* plaque-forming cell response.

Cholera toxin raises the endogenous level of cyclic AMP by stimulating adenyl cyclase activity[17]. The methyl xanthine 3-isobutyl-1-methyl xanthine (IMX) raises the endogenous cyclic AMP level by inhibiting phosphodiesterase activity[18]. Both agents blocked SEA suppression of the *in vitro* anti-SRBC plaque-forming cell response in a manner similar to that observed for dibutyryl cyclic AMP (Table 2). Further, they blocked SEA stimulation of interferon production in mouse spleen cell cultures at concentrations that blocked SEA suppressor activity. Theophylline, also a phosphodiesterase inhibitor, had an effect similar to that of IMX on the suppressor activity of SEA.

Dibutyryl cyclic GMP (10^{-3} and 5×10^{-4} M) did not affect the ability of dibutyryl cyclic AMP (1.4×10^{-4} M) to block the suppression of the plaque-forming cell response by SEA; nor was the effect of dibutyryl cyclic AMP on mitogen stimulation of interferon affected by dibutyryl cyclic GMP. This is further evidence that the phenomena reported here are due to cyclic AMP, and are not influenced by cyclic GMP is this system.

The data are evidence that cyclic AMP, at the concentrations ($2.0\times10^{-5}-2\times10^{-4}$ M) employed here, selectively blocks mitogen induced suppressor cell activity, while not inhibiting T helper cell function, since SRBC is a thymus-dependent antigen[14]. Further, the biological function was associated with inhibition of mitogen induction of interferon in the spleen cell cultures. This suggests that mitogen-induced interferon is associated with mitogen-induced suppressor cell activity, and that the interferon may possibly be a mediator of such activity[11]. In related studies, it has been shown that high concentrations (10^{-3} M) of dibutyryl cyclic AMP can inhibit the yield of interferon from phytohaemagglutinin-stimulated human peripheral leukocytes[5] and from virus- or polyribonucleotide-stimulated mouse L cell cultures[19]. The data presented here show a logical and integrative relationship between cyclic AMP, lymphokine (interferon) activity, suppressor cell activity, and regulation of the immune response.

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Variant strain of rabbits lacking immunoglobulin κ polypeptide chain

The antibody molecule consists of heavy and light polypeptide chains[1]. The light chains are shared by all immunoglobulin classes and they can be divided into two types, κ and λ. The great majority (up to 95%) of rabbit IgG molecules possess the κ type. This polypeptide chain, like many others, shows genetic polymorphism called allotypy[2–5]; the b locus of the rabbit determines allotypes which exist in four allelic forms, namely b4, 5, 6 and 9. They are inherited in Mendelian fashion as codominant genes[3,4] and they have been correlated with amino acid sequences in the constant part of the κ chain, although some authors could associate amino acid differences in the variable part of this chain with certain b locus allotypes[4–7]. The allotypes of the λ chain are determined by an independent c locus (specificities c7 and 21). They do not show Mendelian segregation, at least in our rabbit colony, although they do in certain strains[1,8].

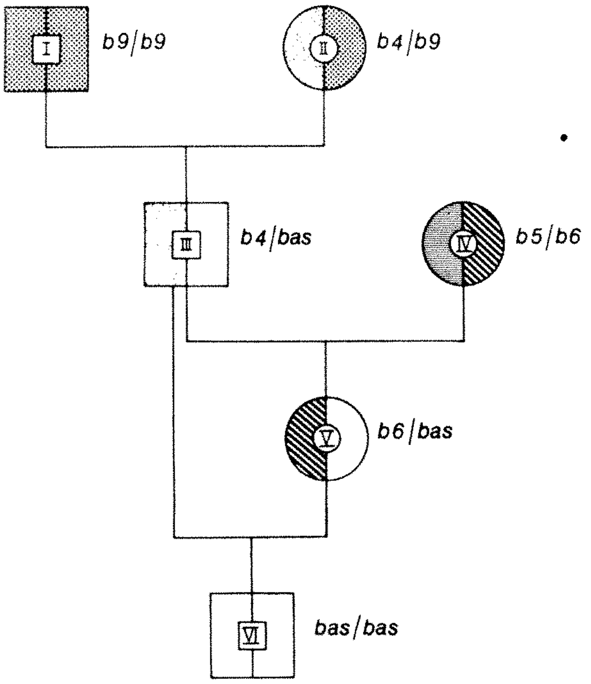
We have established a new rabbit strain, named BASILEA, which derived from a mutant. Individuals of this strain lack the κ polypeptide chain which is replaced almost entirely by the λ type. The mutation, called *bas*, is an allelic form of the b locus.

A male rabbit (Fig. 1, no. III) was born in our colony. He lacked the b9 allotypic marker of the homozygous father (Fig. 1, no. I) who sired 35 normal offspring, all expressing this allotype. Rabbit no. III could have been classified as an illegitimate child. However, parents and offspring of this family have been checked for more than 20 genetic markers of immunoglobulins and all but one (b9) were in agreement with established patterns of inheritance. In addition, rabbit no. III has been tested with 15 anti-b9 antisera in double diffusion with negative results, although the method (see Fig. 2) is able to pick up antigen concentration as low as 10–1 μg ml^{-1}.

Subsequently, rabbit no. III has been outbred and then back-crossed, and the progeny brother-sister mated. Table 1 summarises data from this breeding, and shows that this b allotype negative marker behaves like an allele at the b locus (for example, rabbit no. VI in Fig. 1).

Extensive immunisation[3] with IgG bas of rabbits of various genetic make up did not result in production of anti-allotypic antibodies against b bas as a whole. (It did, however, reveal another allotypic system which is the subject of a separate paper). On the other hand, homozygous *bas/bas* rabbits were capable of making good antibodies against the b4, 5, 6 and 9

Fig. 1 Pedigree of the original bas family.



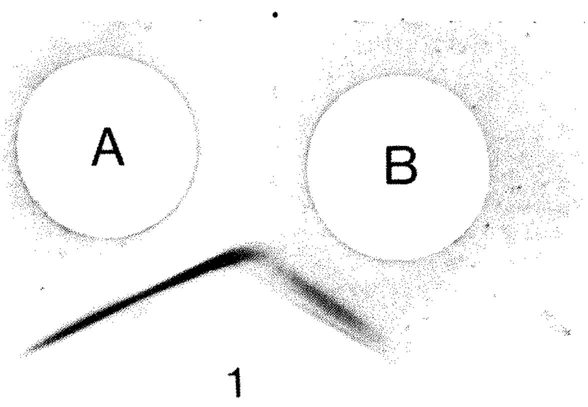


Fig. 2 Double diffusion pattern in agarose showing identity between anti-b9 produced in a standard rabbit (A) and in a bas mutant (B); (1) pool of IgG b9 (1 mg ml^{-1}). Double diffusion tests were performed in a gel composed of 1.5% agarose (A45, Indubiose, L'industrie Biologique Française, S.A.) and 2% polyethylenglycol 6000 in PBS (phosphate buffered saline) at *p*H 7.2. The plates, 1 mm thick, with 5 mm distance between the wells, were developed at 4 °C for 24–48 h. They have been examined unstained or stained with amino black or Coomasie blue. This method allowed us to see the precipitation lines at the antigen concentration as low as 10^{-1} μg ml^{-1}.

Fig. 3 Direct binding of anti-light chain allotypic and anti-isotypic antisera to a pool of Fab bas fragment. Radioimmunoassay: Fab fragments of rabbit IgG have been prepared by papain digestion in a routine fashion and iodinated (^{125}I) using the chloramine T method (50 μg mCi^{-1})[9]. The tests were performed immediately after labelling. The antigen was diluted in 1% ovalbumin made in PBS to give 10,000 c.p.m. per 50 μl. This latter volume contained 8,000 c.p.m. of ^{22}NaCl as volume control. Various amounts of antiserum were mixed and incubated, 2–4 h at room temperature with this aliquot of antigen. After the incubation, 0.1 ml of 10% *Staphylococcus aureus* vaccine, strain Cowan I, has been added[10]. This strain is known to possess large amounts of a so-called protein A which binds Fc of most mammalian IgG classes. The *Staphylococcus* suspension was prepared in PBS containing 0.1% Na azide and 0.2% Nonidet P-40 detergent. This suspension was washed well to remove debris and other impurities of free protein A. After incubation the mixture was centrifuged at 5,000 r.p.m. for 15 min and about 50% of the supernatant removed, then the pellet fraction and the supernatant examined in the γ counter. Percentage binding, including volume control, was calculated according to the equation published previously[11]. We controlled the maximum binding by titrating the antiserum and *Staphylocoocus*. Non-specific binding was within the range of 3%. Tests were carried out in duplicate.

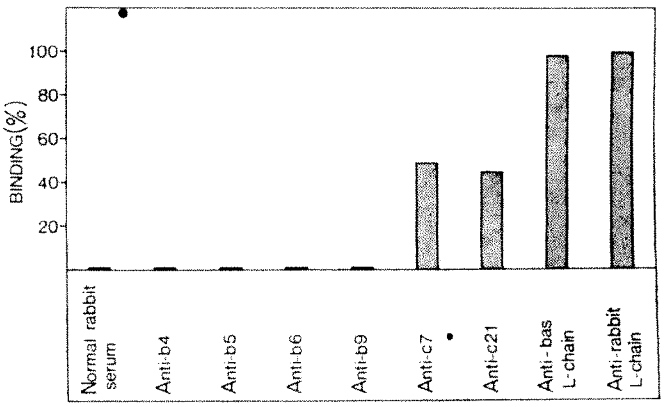


Table 1 Distribution of allotypes in the progeny of crosses involving the *bas* marker

		b allotype of progeny							
Parents	Total	—	i	j	k	ik	jk	χ^2	d.f
bas/*bas* × *bas*/*bas*	16	16 (16)	—	—	—	—	—	—	—
bas/*bas* × b^i/*bas*	70	36 (35)	34 (35)	—	—	—	—	0.057	1
bas/*bas* × b^i/b^i	26	—	26 (26)	—	—	—	—	—	—
bas/*bas* × b^i/b^j	37	—	20 (18.5)	17 (18.5)	—	—	—	0.243	1
b^k/*bas* × b^i/*bas*	31	9 (7.75)	9 (7.75)	—	5 (7.75)	8 (7.75)	—	1.387	3
b^k/*bas* × b^k/*bas*	6	1 (3)	—	—	5 (3)	—	—	1.500*	1
b^k/*bas* × b^k/b^k	1	—	—	—	1 (1)	—	—	—	—
b^k/*bas* × b^i/b^k	6	—	1 (1.5)	—	1 (3)	4 (1.5)	—	3.417*	2
b^k/*bas* × b^i/b^i	47	—	23 (23.5)	—	—	24 (23.5)	—	0.021	1
b^k/*bas* × b^i/b^j	41	—	11 (10.25)	9 (10.25)	—	10 (10.25)	11 (10.25)	0.268	3
Total	281							6.893 $P = 0.86$	12

b^i, b^j and b^k represent various alleles at the b locus. For example, b^i/b^j are heterozygous and may be b^4/b^5 or b^4/b^6 or b^5/b^6 . . . etc. Phenotypes i, j and k include also *bas* heterozygotes. The expected values, shown in brackets, are used for the χ^2 calculation.

*These values are calculated including the Yates' correction for continuity.

allotypes. It is worth noting that such an anti-b4 antiserum cross reacts strongly in double diffusion tests in gel, with b5 and b6. What is even more important, the bas rabbits can produce specific anti-b9 antiserum (see Fig. 2). This is convincing evidence that the ancestral b9 allotype was absent from the *bas* mutant.

To confirm the results obtained by double diffusion in gel that the κ chain allotypes were absent from IgG bas, direct binding of various allotypic and isotypic antisera to Fab bas was carried out. Figure 3 illustrates these results. None of the anti-b allotype antisera bound Fab bas in a detectable amount. On the contrary, anti-c7 and anti-c21 antisera, specific for λ chain allotypes, bound up to 95% of bas (50% and 45% respectively). Guinea pig anti-bas light chain and swine anti-rabbit light chain (Nordic Immunology) bound bas up to 100%. Anti-κ light chain antisera were excluded from this test as non-specific (contaminated with anti-λ antibodies).

Guinea pigs were immunised with λ light chain from rabbits suppressed for the b locus allotype[12–14], and with the bas light chain. Figure 4 illustrates that the bas chain is either identical with or very closely related to the light chain of the suppressed rabbits.

This observation has also been confirmed by the inhibition of direct binding of anti-bas light chain to Fab bas (Fig. 5). Fab fragments were used as inhibitors (light chain preparations gave similar results). The homologous Fab fragment (bas) inhibited the binding 100%, whereas the Fab of suppressed rabbits showed biphasic curves reaching also almost 100% inhibition eventually. Altogether only 50% of this light chain (as Fab) binds anti-c7 and anti-c21 antibodies, and therefore may contain determinants in a concentration different from that of the homologous Fab bas. When Fab b9, which contained 10% non-b9 (λ chain), was used as inhibitor, a pattern similar to the homologous one was observed but the curve was shifted by one order of magnitude. The b9 preparation came from rabbits closely related to the strain BASILEA. The controls with Fab b4 and b5 showed different inhibition curves, indicating much less similarity between bas and b4 or b5. This can be explained by the presence of λ chain in these preparations. Nevertheless, weak cross-reactivity between κ and λ chains cannot be ruled out. The radioimmunoassay revealed in addition that the preparations of the bas chain and of the light chain of suppressed rabbits has about 5% of either subtype of κ or subtype of λ[15].

Preliminary immunochemical data (in collaboration with Dr J-C. Jaton) show that the amino acid composition of the bas light chain resembles the λ type. As judged by similarities, the peptide maps of the light chains[16,17] fall into two groups; group I: b9, bas light chain and that of suppressed rabbits (almost entirely λ), group II: b4, b5 and b6 (all three chain preparations almost entirely of the κ type).

To see how the bas mutation influences the expression of the b locus, various Fab preparations from rabbits, homozygous or heterozygous for the b locus were tested for binding to anti-b allotype sera. The antisera used were strictly specific; none of them bound Fab bas but they reacted with 90–95% of their corresponding Fab. The same was true when Fab preparations from animals heterozygous for bas and the corresponding *b* allele were tested. Fab b4-5 from heterozygotes show the

Fig. 4 Double diffusion pattern in agarose showing identity or close similarity between anti-bas light chain (A) and anti-light chain from suppressed rabbits (B) made in guinea pig; (1) light chain from rabbits suppressed for the b-locus 1 mg ml^{-1}. The test was performed as described in Fig. 2. The precipitation was overdeveloped to see also minor components. When bas light chain was used as antigen analogous results were obtained.

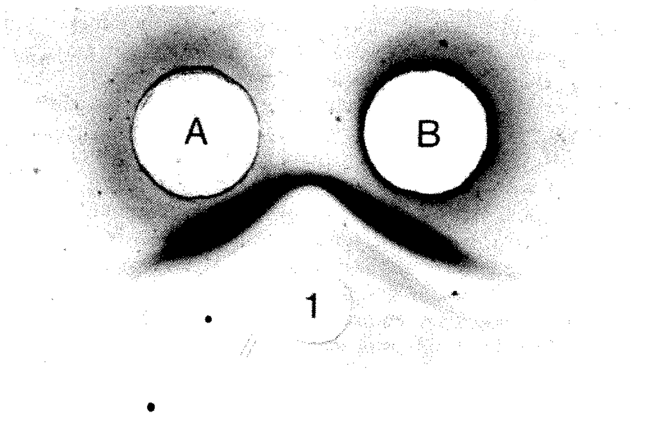
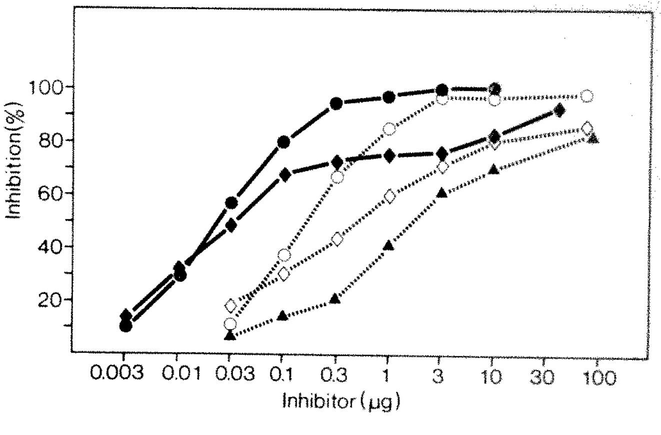


Fig. 5 Inhibition of binding of anti-bas antiserum to Fab bas by various Fab fragments. The test was performed as described in Fig. 3 but different amounts of inhibitor were added to the antigen before application of the antiserum. A serum concentration was used to get 50% binding without inhibitor. Percentage inhibition was calculated according to a published formula[11]. Each point represents the mean of quadruplicates. ●, bas; ◆, suppressed for b5; ○, b9; ◇, b5; ▲, b4.



expression of both alleles as expected[4]. By analogy, with the rabbits heterozygous at the b locus and suppressed[14] for one allele only, the heterozygotes *b4/kas* and so on maintain a normal κ: λ ratio, that is, one 'κ chromosome' is enough to regulate this ratio.

Rabbits of the strain BASILEA are healthy and have normal level of IgG unlike the few cases described in man[18,19]. They produce good antibodies to rabbit allotypes and idiotypes, against sheep erythrocytes, dinitrophenol (DNP), and *Proteus*, *Pneumococcus* and *Streptococcus* vaccines. Immunisation with the group A-variant streptococcal vaccine (in collaboration with Dr D. G. Braun) gave a high proportion (8 out of 10 individuals) of restricted immune response[20] as judged by microzone electrophoresis and isoelectric focusing. The antibodies eluted from the bacterial cell wall and tested serologically proved to be of the λ type.

In conclusion, we may say that the *bas* variant of the rabbit arose by spontaneous mutation affecting the b locus of the chromosome region coding for the κ polypeptide chain or an essential part of it. Deletion seems to be the most likely mechanism, although many other explanations could be considered. The BASILEA strain resembles rabbits suppressed completely for the b locus allotypes, and it offers immense experimental opportunities.

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Lymphocytes with T-cell markers cooperate with IgG antibodies in the lysis of human tumour cells

THYMUS-DERIVED (T) lymphocytes from animals immunised with syngeneic or allogeneic tumour cells are well known to kill specifically target cells *in vitro*[1]. The as yet unidentified antigen recognition structure on T lymphocytes is not deemed to be a conventional type of immunoglobulin. In contrast to this kind of cell-mediated cytolysis another cellular killing mechanism has been described, called antibody-dependent cell-mediated cytolysis (ADCC), in which cells bearing the Fc receptor (FcR) cooperate with humoral antibody in the cytolytic action[2,3]. The nature of the main lymphoid effector cells (K cells) involved in the latter system is still ill-defined. They are supposed not to be T lymphocytes. Recently however, Lamon *et al.* reported on mouse thymocytes which were able to lyse MSV-induced tumour cells using IgM antibodies[4]. In this report we present evidence that human lymphocytes bearing the T marker in collaboration with homologous or autologous antibodies can efficiently kill tumour cells *in vitro*.

The data were obtained with an ADCC system[5,6] consisting of cultured melanoma cells as target cells, different preparations of human peripheral blood lymphocytes as effector cells, and anti-melanoma cell antisera obtained from melanoma patients. The cytolytic activity of lymphocytes was measured by the ^{3}H-proline release test as described previously[7]. Antibodies increasing the spontaneous cell-mediated cytolysis (CMC) of melanoma cells by more than 100% were detected in 17 out of 87 patients. Immunoglobulins were isolated from patients' sera by affinity chromatography using insolubilised antibodies rendered monospecific for heavy chain determinants of either IgG, IgM or IgA. Non-adherent lymphocytes were prepared as described previously[7]. T lymphocytes defined as cells having receptors for sheep erythrocytes (SRBC), were purified by two different procedures: either by passing the cells through IgG anti-IgG Degalan^R-bead columns according to Golstein *et al.*[8] or by density gradient centrifugation as spontaneous rosettes with SRBC (E-RFC)[5]. The various lymphocyte preparations were characterised by surface markers as depicted in Table 1.

As can be seen from Fig. 1*a* the passage of lymphocytes through an IgG anti-IgG bead column resulted in a complete loss of spontaneous cytotoxicity. However, the capacity of column-filtrated cells to cooperate with anti-tumour antibodies was not affected when tested in the long term ^{3}H-proline release assay. Furthermore, these cells still responded to mitogens such as PHA and concanavalin A indicating functional integrity (Table 1). When—as a control—cells were passed through a FCS-coated Degalan column, neither the CMC- nor the ADCC-activity was reduced. These findings obtained in eight independent experiments were rather unexpected since almost no FcR-bearing cells could be detected immediately after column fractionation when tested by a sensitive radioautographic method using ^{125}I-labelled aggregated IgG (^{125}I-agg. IgG) of defined size[9] (Table 1).

Because ADCC is thought to depend on the presence of FcR-bearing cells the question arose whether Fc receptors and ADCC activity were generated *de novo* during the 40 h cocultivation with target cells. Therefore, column fractionated lymphocytes were incubated together with unlabelled tumour cells for 6, 12, and 48 h. After various periods of incubation the FcR-bearing cells were enumerated and ADCC was assessed in a short-term 6 h ^{3}H-proline release assay. As demonstrated in Table 2 the percentage of FcR-bearing cells increased with time of cocultivation, reaching approximately the pre-filtration value after 48 h. No ADCC activity could be detected immediately after the column passage, independent of the effector cell–target cell ratio used (Fig. 1*b*). However, significant ADCC reappeared after 12 to 24 h of cocultivation with either syngeneic or allogeneic melanoma cells, and increased further up to 48 h. In some experiments (for example experiment 2, Table 2) the generated ADCC activity considerably exceeded the prefiltration value. In control experiments where lymphocytes were cultured without tumour cells under otherwise identical conditions very few FcR-bearing cells could be detected up to 48 h and ADCC remained marginal when compared with that of cocultured cells. Thus, the increase of the number of FcR-bearing cells and ADCC activity during cocultivation may not be due to deblocking of FcRs occupied by IgG or complexes released from the column. This assumption is supported by additional observations (data not shown): first, when ^{125}I-labelled goat anti-human IgG or ^{125}I-labelled human IgG (at least 100 μCi per column) were used for the preparation of the IgG anti-IgG column, neither by direct counting in a gamma counter nor by radio-autography could a significant radioactivity be detected on the passed cells. Second, the proportion of FcR-bearing cells after column filtration did not increase after trypsin treatment. In addition, unspecific retention of cytotoxic effector cells on protein-coated Degalan beads was rendered unlikely, since FCS-coated control columns did not reduce the proportion of FcR-bearing cells. The difference in cell yield, however, between both columns

Table 1 Characterisation of purified lymphocytes immediately after filtration through IgG anti-IgG bead columns or FCS-coated control columns and separation of E-rosette forming cells

Lymphocyte preparation	FITC-anti-Ig-positive cells (%)	^{125}I-aggregated IgG-binding cells (%)	^{125}I-E-rosette forming cells (%)	monocytes* (%)	^{3}H-thymidine incorporation (c.p.m.) at optimal concentrations of PHA (10 μg ml^{-1})	^{3}H-thymidine incorporation (c.p.m.) at optimal concentrations of Con A (25 μg ml^{-1})
Non-adherent lymphocytes	5.2 ± 1.7	10.0 ± 2.8	83.0 ± 5.5	1.9 ± 0.6	53,840 ± 1924	26,920 ± 1793
IgG anti-IgG† column filtrated lymphocytes	<0.1	<0.1	96.2 ± 3.1	<0.2	17,498 ± 1302	6,730 ± 91
FCS-column† filtrated lymphocytes	—	8.1 ± 2.4	80.3 ± 2.1	—	—	—
Pelleted E-rosette forming cells	0.5 ± 0.2	3.5 ± 0.7	96.8 ± 2.7	<0.2	20,190 ± 1744	15,479 ± 998

Results are means ± s.d. of five independent experiments, except the PHA and con A stimulation where the data of one representative experiment are given. Preparation of effector cells from human peripheral blood and determination of surface markers was performed as described previously[5,7,9]. SRBC were lysed by short (20–40 s) hypotonic treatment with distilled water. ^{3}H-thymidine incorporation in con A and PHA-stimulated cultures was determined using 1×10^6 lymphocytes, cultured in 1 ml RPMI 1640 supplemented with 10% human AB-serum for 48 h in the presence of various concentrations of PHA-P (DIFCO Laboratories) (1–10 μg ml^{-1}) and con A (Pharmacia Fine Chemicals) (0.1–25 μg ml^{-1}). After 40 h of culture 1 μCi ml^{-1} ^{3}H-thymidine was added for an additional 8 h. Results are expressed as mean c.p.m. of triplicate cultures ± s.e.

* Percentage of monocytes was estimated according to reactions for N-AS-Acetate-Esterase and Peroxidase.

† Recovery of cells from the column: 50–60%.

‡ Recovery of cells from the column: 80–85%.

** Radioactivity incorporated in unstimulated cultures 673 ± 312 c.p.m.

cannot be fully explained by retention of cells bearing detectable FcRs and/or S-Ig (Table 1).

More than 95% of the column fractionated cells proved to be E-RFCs. Since a considerable portion of the FcR-bearing lymphocytes generated *in vitro* also bound SRBC, it was assumed that these cells were derived from the original T-cell inoculum. Such double marker cells are also present in the peripheral blood as originally described by Dickler *et al.*[10] Thus lymphocytes were isolated as E-rosettes and found to contain about 3.5% of ^{125}I-agg. IgG-binding cells, most of them simultaneously binding SRBC (Table 1). These purified E-RFCs displayed ADCC activity comparable with unseparated lymphocytes in a 6 h ^{3}H-proline release assay without prior cultivation on tumour cells (Fig. 1*c*).

The antibodies which induce cytolytic effects with the subset of lymphocytes described above could be confined to the IgG class as demonstrated in Table 3. IgM or IgA fractions of the antisera tested so far were not active in ADCC.

If the binding of SRBC is considered a valid marker for human T lymphocytes, the data presented strongly suggest that a subpopulation of T lymphocytes is able to induce lysis of tumour cells with the help of IgG antibodies. So far, only peripheral T lymphocytes showed this effect, whereas thymocytes were inactive. The mechanism of the *in vitro* induction of cooperative cytotoxicity is unknown. The time kinetics suggest that activation by lymphocyte–tumour cell contact may lead to a rapid reappearance of Fc-receptor activity on the effector cells, which in turn might increase ADCC activity. In agreement with the data presented are the observations of Biberfeld and Perlmann[11] describing a considerable proportion of lymphocytes bearing T-cell marker in a cytolytic plaque assay. Receptors for the Fc-portion of either the IgG[12-15] or the IgM[4,15] molecule have been described for various T-cell subpopulations. The finding that spontaneous cytolytic activity is lost by passage of the lymphocytes through IgG-anti-IgG columns, also described by others[16], suggests that FcR- and/or S-Ig bearing cells are effector cells in spontaneous cytotoxicity. Recent findings in our laboratory (J. G. Saal *et al.*, in preparation) together with the data presented here, point to the possibility that T lymphocytes armed *in vivo* by humoral IgG

Table 2 Appearance of aggregated IgG binding and ADCC activity during cocultivation of human lymphocytes bearing T markers with allogeneic and syngeneic melanoma cells

Expt	Cultivation of purified lymphocytes	%CTL in a 6-h ADCC assay: Before column filtration	%CTL: Period of cultivation after column filtration (h) 0	12	48	% ^{125}I-agg.IgG-binding cells: Before column filtration	% ^{125}I-agg.IgG-binding cells: Period of cultivation after column filtration (h) 0	12	24	48
1	With allogeneic tumour cells	17 ± 4	0 ± 0	4 ± 1	12 ± 1	5.5	0.2	0.8	1.5	2.5
	Without tumour cells			0 ± 0	3 ± 2			0.3	0.1	0.2
2	With allogeneic tumour cells	13 ± 1	0 ± 0	8 ± 1	53 ± 2	5.5	0.1	3.2	–	5.0
	Without tumour cells			3 ± 2	8 ± 3			0.1	–	0.1
3	With syngeneic tumour cells	10 ± 1	2 ± 1	6 ± 2	15 ± 2	6.7	0.5	1.4	1.8	4.7
	Without tumour cells			1 ± 1	2 ± 1			0.3	0.2	0.3

Lymphocytes from melanoma patients were depleted for Fc-receptor- and S-Ig-bearing cells by filtration through IgG anti-IgG bead columns and cultivated either alone or together with 1×10^4 syngeneic or allogeneic melanoma tumour cells in Falcon-Microtest II plates. The cells were collected at the intervals indicated and tested for either ADCC activity in a 6-h ^{3}H-proline release test (as described in legend to Table 3) or uptake of ^{125}I-agg.-IgG by radioautography. Effector cell–target cell ratio = 50:1.

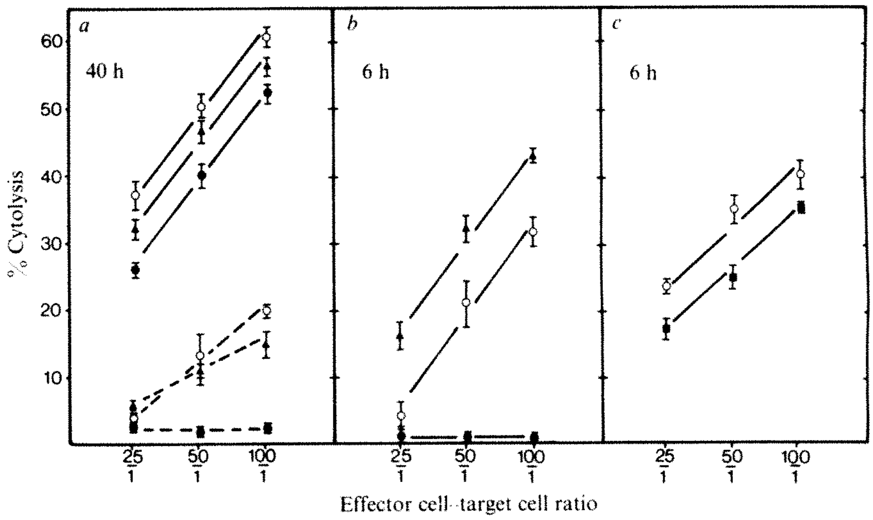

Fig. 1 Spontaneous (CMC) and antibody dependent (ADCC) cytolytic activity of different lymphocyte preparations. Open circles (○) indicate the cytolytic activity of cells before column filtration or E-rosette centrifugation (non-adherent lymphocytes). ●, Cells after passage through IgG anti-IgG columns; ▲, cells after passage through FCS-coated control columns; ■, pelleted E-RFCs. CMC is given by the dashed lines and ADCC by the solid lines. Cytolytic activities were measured either in a 6-h (expt. *b*,*c*) or a 40-h (expt. *a*) ^{3}H-proline release test. The test was performed as described in legend to Table 3. Effector cell–target cell ratios of 25/1, 50/1 and 100/1 were tested. Results are means of triplicate tests ± s.e. In the 6-h assays depicted in Fig. 1*b*, *c* no measurable CMC was detected.

Table 3 Cytolytic activity of lymphocytes before and after filtration through IgG anti-IgG bead columns: cooperative effect with antibodies directed against melanoma cell membrane

Antiserum added	Cytolysis (%) in a 40-h ^{3}H-proline release test with Non-adherent lymphocytes	Cytolysis (%) in a 40-h ^{3}H-proline release test with Lymphocytes after IgG anti-IgG column fractionation
No serum (CMC)	17±1	0±0
Whole serum	44±1	40±1
IgG fraction	39±1	39±1
IgM fraction	14±2	2±1
IgA fraction	11±3	n.t.

CMC was measured by the ^{3}H-proline release test as previously described in detail[7]. $5 \times 10^6 - 1 \times 10^7$ mel-Ei-78 tumour cells labelled 24 h with 50 μ Ci ^{3}H-proline (500–1,000 mCi mmol^{-1}) were distributed into Falcon Microtest-II plates (1×10^4 per well). 5×10^5 effector lymphocytes were added in RPMI 1640 with 10% human AB serum. ADCC was assessed by adding a human anti-melanoma antiserum to the CMC system at a final dilution of 1:6. IgM or IgA was isolated from the antiserum by affinity chromatography with sepharose-coupled antibody previously rendered monospecific for the IgG-IgM- or IgA-heavy chains.

ADCC is defined as antibody-induced cytolysis superimposed on spontaneous CMC. The antiserum without lymphocytes never showed more than 4% cytolysis (CTL). After 6–40 h of incubation at 37 °C the total content of each well was collected on glass filters by use of an automatic harvesting device. The radioactivity retained on the filter was determined in a liquid scintillation counter.

CTL was calculated by:

$$\% \text{CTL} = 100 - \frac{\text{c.p.m. test sample}}{\text{c.p.m. medium control}} \times 100$$

Results are means of triplicate tests ± s.e.
n.t., not tested.

antibodies are involved in spontaneous cytotoxicity.

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Cμ4 domain of IgM has cytophilic activity for human lymphocytes

WE have isolated and identified two intact Cμ4-domain fragments from a monoclonal IgM protein. They differed only in their carbohydrate content, having the same polypeptide chain. They were separated from each other and from Fcμ monomer by molecular exclusion and affinity chromatography (insolubilised concanavalin A[1]) of mildly reduced and alkylated Fc$_5\mu$ produced by the method of Plaut and Tomasi[2]. The Cμ4 fragment containing carbohydrate was designated Cμ4(+) while the carbohydrate-poor domain was called Cμ4(−). These two fragments were identified as Cμ4-domains from their amino acid sequences, amino acid compositions, molecular weights (14,700) and antigenic characteristics. Both fragments originate from tryptic cleavage at Lys-445 of the μ chain (IgM(Ou) numbering[3]) and could not be further fragmented by extensive reduction and alkylation[4]. The domain hypothesis of Edelman *et al.*[5] proposes that each homology region of the immunoglobulins evolved to perform independent biological effector functions. Hurst *et al.*[6] have shown that fragmented Cμ4 domain will fix activated Cl(Cl) and will also activate proenzyme Cl to Cl'. Moretta *et al.*[8] have demonstrated receptors specific for IgM on human T lymphocytes, and this has been confirmed by McConnell and Hurd[9]. We now report that these receptors are specific for the Fc part of IgM (Table 1) and furthermore that the Cμ4 domain is involved in this affinity reaction in a dose-dependent manner (Fig. 1).

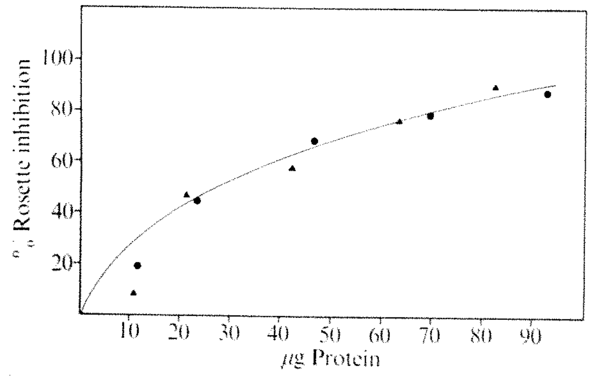

Fig. 1 Dose-dependent inhibition of EA(IgM) rosette formation by C 4(+) protein. Methods used were the same as that described in Table 1. ●, Experiment 1; ▲, experiment 2.

Several workers have concluded that the classical pathway of complement activation is mediated by the Cγ2 (ref. 13) region of IgG (Table V, ref. 14). In the case of cytophilic reactions, however, the situation is not so well defined; Cγ2, Cγ3 or intact Fc region being implicated (Table V, ref. 14). Our results show that Fcμ (that is Cμ3–Cμ4) and Cμ4 fragment inhibit EA(IgM) rosette formation to approximately the same extent, and it must therefore be deduced that the Cμ3 domain does not contribute significantly to this activity. In contrast to Fcγ, minimal non-covalent interactions occur in Fcμ (ref. 15), and as a result the protein exists mainly as a monomer in solution. This is important because both Fcμ and Cμ4 inhibit rosette formation quite efficiently (Table 1), indicating that the Fcμ receptor does not require antigen binding to or a polymeric form of IgM in order to fulfil its unknown function. Two biological functions—complement fixation[6] and cytophilic activity—have now been attributed to the Cμ4 domain of IgM.

Table 1 Inhibition of EA(IgM) rosette formation by IgM and some of its fragments

Inhibitor	Concentration (mg ml^{-1})	Inhibition of EA(IgM) rosettes (%) exp. 1	exp. 2
None	—	0	0
IgG	9.9	2.2	0
IgM	0.52	81.9	90.6
Fc5μ	0.50	97.8	96.1
Fcμ	0.52	53.6	58.6
Fabμ	4.1	0	0
Cμ4(+)	0.42	47.8	46.1
Cμ4(−)	0.70	65.2	59.4

The formation (and inhibition) of IgM-dependent rosettes (EAIgM-RF) was carried out as described by Moretta *et al.*[8] Rabbit antisera containing mainly IgM antibodies to ox erythrocytes were raised according to Mayer[10] and the fraction containing IgM was isolated by molecular exclusion chromatography on Sephadex G-200. IgG was purified by DEAE–cellulose chromatography of Cohn fraction II and IgM, $Fc_5\mu$, Fcμ and Fabμ as previously described for normal IgM[11]. The protein concentration of the inhibitors tested was determined by the Folin Ciocalteau method[12] using recrystallised bovine serum albumin as standard. Three hundred lymphocytes were counted for each test and only those cells with three or more adhering EA(IgM) were scored as rosettes.

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Common antigens and male-female recognition in plants

THE question of how the female stigma of the plant recognises a pollen grain carrying a compatible male gamete from all the possibilities presented to it by air currents and visiting insects is an intriguing problem[1]. When pollen is recognised at the stigma surface, a mixture of components, including proteins and glycoproteins, are released from cavities in the pollen walls[2,3]. These components have been partially characterised[4,5] and include those essential to the recognition process[6]. The pollen wall components flow over and bind to the stigma surface which is covered by a secreted sticky coating[7]. The binding of specific recognition factors determines the success of a mating[8]. We have attempted to identify factors involved in pollen-stigma recognition, using immunochemical methods with rabbit antisera to pollen and stigma surface components. The major antigen of the stigma surface shows immunological identity and has the same molecular weight as an antigenic pollen wall component. This stigma antigen is also partially identical with components of other tissues of the same plant. Absorption experiments show that the cross-reacting antibodies can be removed from the antisera, leaving antibodies which interact only with the immunising antigen. This raises the question whether plant cells, like animal cells[9], are specified according to their family, species, organ and tissue by surface determinants.

Antisera were raised in rabbits to both stigma surface extracts and pollen wall components of *Gladiolus*

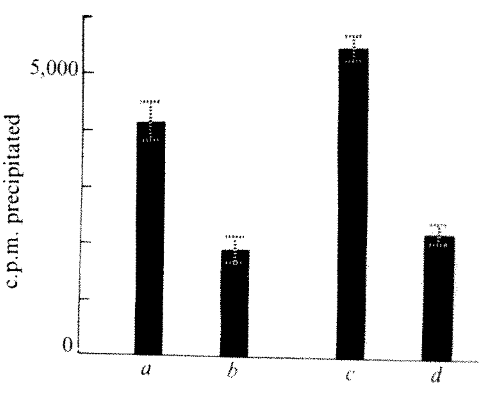


Fig. 1 Immune precipitation of ^{125}I-pollen and stigma antigens of *Gladiolus* by homologous and heterologous antisera. A double antibody precipitating system was used: to 200 μl of radio-iodinated pollen or stigma extracts (10^6 c.p.m.) were added 100 μl of a 1 : 20 dilution of rabbit antiserum. The precipitate was developed by addition of 200 μl of a 1 to 4 dilution of goat antiserum to rabbit IgG followed by incubation for 1 h at 37 °C and 15 h at 4 °C. Data given are ^{125}I-radioactivity associated with precipitates which were washed twice by centrifugation. *a*, ^{125}I-pollen + anti-pollen serum; *b*, ^{125}I-pollen + anti-stigma serum; *c*, ^{125}I-stigma + anti-stigma serum; *d*, ^{125}I-stigma + anti-pollen serum.

gandavensis cv[10]. Stigma antigens were prepared by washing receptive stigmas with 0.05 M Tris buffer, *p*H 8.4, 0.15 M NaCl and 0.001 M $CaCl_2$, and dialysing and freeze drying the material washed from the surface. Pollen antigens were allowed to diffuse from the wall cavities for 10 min in the same buffer and the filtrate was dialysed and freeze dried.

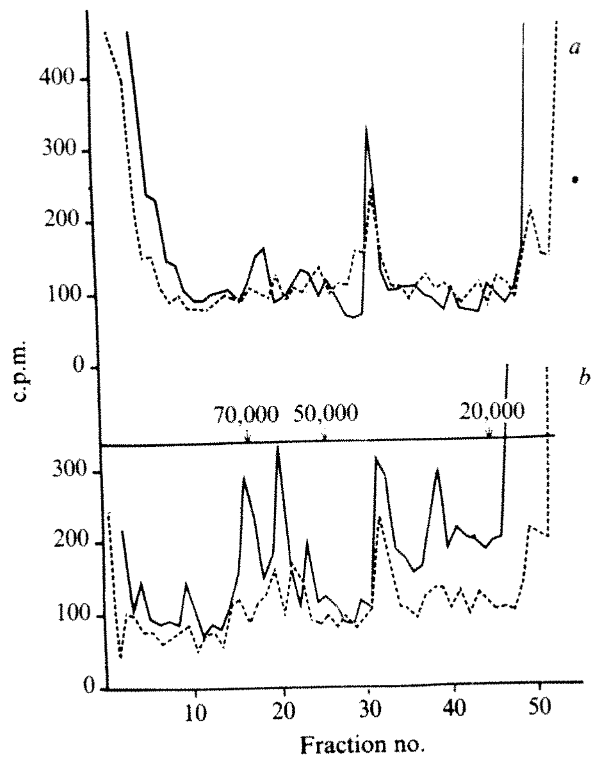
Samples of both stigma and pollen antigens were labelled with ^{125}I by the lactoperoxidase method[9] and incubated with both the homologous and heterologus antisera. The immune precipitates were collected, washed and counted. The results (Fig. 1) show specificity of precipitation in both the homologous stigma–antistigma and the pollen–antipollen systems, as well as some cross reactivity in the heterologous systems.

The washed immune precipitates were dissociated and the components were separated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. The gels were cut into 2-mm slices which were counted individually. The distribution of radioactivity is shown in Fig. 2. These patterns show a major antigen in the stigma surface preparation, molecular weight ~40,000, and this cross reacts with antisera raised to pollen from the same plant. The reciprocal experiment confirms this: pollen contains seven major antigens, of which that of molecular weight ~40,000 cross reacts with antisera raised to the stigma surface.

In gel immunodiffusion experiments (Fig. 3), antisera to stigma surfaces show two bands, one of which shows partial identity with a petal component and a pollen wall component. Of the five precipitating antigens of pollen, two are common to both corm and petal, and the slower diffusing of these is also common with the stigma. In addition, petal also contains two other components which form precipitin bands with antisera to pollen, and one of these is also present in corm.

Absorption of antisera to stigma surfaces with petal antigen removed the capacity of the serum to precipitate the petal antigen, but did not apparently affect the capacity of the serum to precipitate either the pollen or the stigma antigens. Likewise, the absorption of the antisera with pollen antigen removed the capacity of the serum to precipitate the pollen antigen, but the absorbed antisera retained the capacity to precipitate the stigma antigens. Stigma-absorbed antisera would not precipitate with either petal, pollen or stigma antigens.

Fig. 2 Analysis of ^{125}I-stigma (*a*) and pollen (*b*) antigens of *Gladiolus* on SDS polyacrylamide gels after precipitation of homologous antisera (solid line) and heterologous antisera (broken line). Immunoprecipitates were dissociated in 200 µl of Tris buffer, *p*H 6.8, containing 3% SDS and 5% β-mercaptoethanol, and run on SDS polyacrylamide gels as described by Laemli and Favre[16]. The gels were sliced and radioactivity was determined using a Packard autogamma counter.



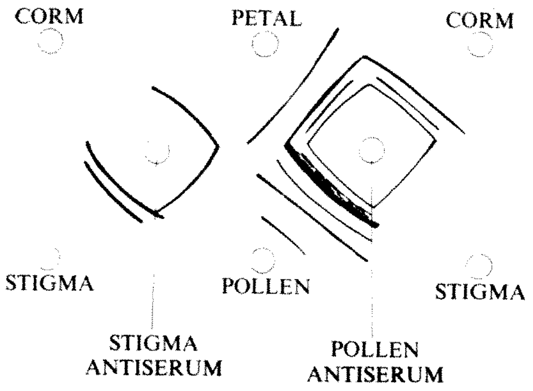


Fig. 3 Immunodiffusion of *Gladiolus* tissue antigens against antisera raised to stigma surface preparations and pollen wall components. Agar gel immunodiffusion plates were run using antigens at 10 µg ml^{-1} in 0.15 M NaCl and whole antisera. Antigens were prepared by homogenising the tissue (0.25 g fresh weight per ml of 0.05 M Tris, *p*H 8.5, 0.15 M Na Cl, 0.001M $CaCl_2$) and dialysing and freeze drying the supernatant.

One explanation for these results is that stigma surface determinants in this system are partially identical with determinants of other tissues of the same plant. It may be that variation in the degree of identity of this component in pollen and stigma controls the positive recognition in the pollen–stigma system. This conforms with suggestions[12–14] that the recognition genes in self-incompatible plants may specify identical products in stigma and pollen. Several groups of stigma surface components have been described[8], but their individual antigenicity is not known. It is, however, possible that some of the antigens are isozymes of hydrolytic enzymes common to both sites[15].

The finding that a closely related determinant is present in petals, and the observation of antigens common to pollen and other tissues of the same plant, may indicate that male-female recognition factors are part of a wider system of recognition factors involved in such basic functions as cell growth and differentiation, which are reflected in economically important problems such as scion–rootstock incompatibility in fruit trees and other horticultural crops.

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Solubilisation of phospholipid membranes by human plasma high density lipoproteins

HUMAN plasma high density lipoproteins (HDL) seem to have a protective effect against the development of atherosclerosis[1,2]. Glomset proposed that unesterified cholesterol diffuses from cell membranes into the HDL particle following esterification of cholesterol in HDL by the action of plasma lecithin: cholesterol acyltransferase[3]. In tissue culture experiments, HDL[4] and complexes of its apoproteins with phospholipids[5] removed cholesterol from arterial smooth muscle cells, and the principal HDL apoprotein, apo-A1, adsorbed to and removed phospholipid from Landschutz ascites cells[6]. We show that intact HDL releases part of its apoprotein when incubated with synthetic phospholipid membranes, leading to the formation of soluble, discoidal phospholipid–apoprotein complexes. The residual apoprotein-deficient HDL particles seem to undergo fusion.

The human plasma HDL are spherical particles, 95–105 Å in diameter, consisting of a polar shell of phospholipid head groups and apoproteins surrounding a core of apolar lipids[7,8]. The protein moiety comprises about 60% apoprotein-A1 (molecular weight 28,331)[9] and 30% apoprotein-A2 (molecular weight 17,380)[10]. Studies of HDL by differential scanning calorimetry have shown that apo-A1 is released from HDL following heating to 100 °C, giving rise to a characteristic reversible thermal denaturation of lipid-free apo-A1[11,12]. This transition with midpoint temperature at 54 °C has been used to monitor release of apo-A1 from HDL (Fig. 1). By heating HDL, cooling, then reheating to progressively higher temperatures, the fraction of apo-A1 released with each increment in temperature was calculated from the enthalpy of the transition at 54° C. These experiments indicate that almost all of the apo-A1 of each HDL preparation can be released in lipid-free form between 55 and 80 °C. Between 80 and 95 °C cholesterol ester is liberated from HDL, which can be demonstrated on cooling by the appearance of reversible liquid crystalline transitions between 11 and 33 °C (Fig 1*g*), and the presence of smectic liquid crystals by polarising microscopy[13]. With each HDL preparation more than 90% of the apo-A1 was released before cholesterol ester liquid crystalline transitions could be demonstrated. Since domains of cholesterol ester as small as 1,000 molecules (as in low density lipoprotein) show liquid crystalline transitions[14], HDL partially depleted of apo-A1 must exist as a stable microemulsion containing small domains of cholesterol ester, probably fewer than 1,000 molecules.

Apo-A1, released from HDL by heating, could be isolated by preparative ultracentrifugation. HDL was heated to 70 °C for 15 min, cooled and centrifuged at d (density) 1.25 g ml^{-1}.

The infranatant contained 5–15 times more lipid-free (that is, containing less than 1% phospholipid) protein than control, unheated HDL preparations, and when examined by Sephadex G-200 chromatography and polyacrylamide gel electrophoresis, the protein consisted of >90% apo-A1. The supernatant was optically clear and by electron microscopy (EM) contained spherical particles of diameters 90–180 Å (mean ± s.e.m. = 145 ± 3.1 Å, n=90), which were significantly larger than those present in control preparations (91 ± 2.8 Å (n=100). The larger particles present in the supernatant probably arose from fusion of HDL particles secondary to the loss of amphiphilic apoprotein molecules. In experiments where HDL was heated to a series of temperatures above 55 °C, an increasing amount of pure apo-A1 was recovered in the infranatant between 60 and 85 °C, in quantities corresponding to the increasing enthalpy of the 54 °C transition in the differential scanning calorimetry experiments (Fig. 1).

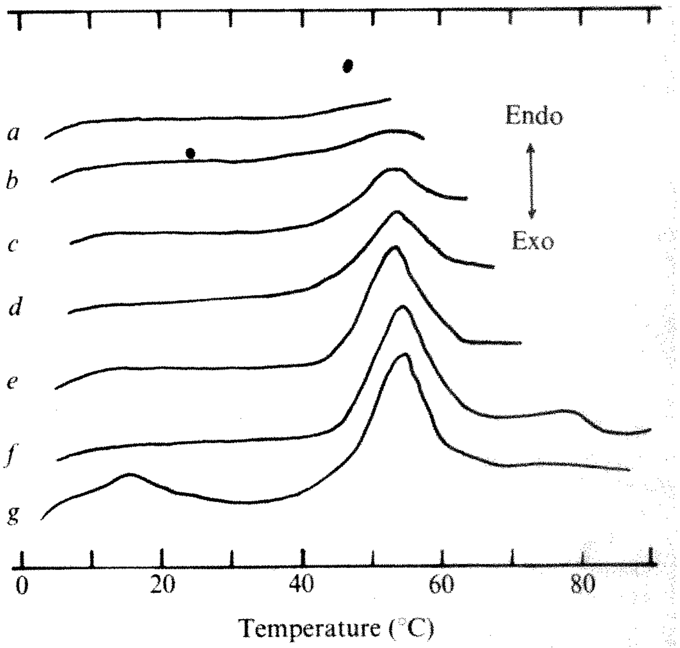


Fig. 1 Differential scanning calorimetry tracings of the thermal disruption of HDL. Human plasma HDL was isolated from the blood of normal fasting male donors by ultracentrifugal flotation between 1.063 and 1.21 g ml^{-1} as previously described[12]. The lipoprotein samples were concentrated to 10–30% solids by vacuum dialysis. Differential scanning calorimetry experiments were performed on a Perkin Elmer DSC-2B, calibrated with cyclohexane and indium. Experiments were performed in 75 λ sealed stainless steel pans at heating rates of 2.5 or 5 °C min^{-1}. HDL was heated to progressively higher temperatures with 5 °C increments on each heating run, and cooled at 10 °C min^{-1}. The heating runs to 75, 80 and 85 °C were identical to *e*. Experimental results were independent of lipoprotein concentration. In experiments where apo-Al was heated repeatedly to the same temperature, above 55 °C, there was an increase in the endotherm at 54 °C, on the second heating run (by 10–40%), the third heating run (5–10%), but not on the fourth and subsequent heating runs. Repeated heating to temperatures below 55 °C did not result in liberation of apo-Al. The low temperature transition (Fig. 1*g*) is attributed to cholesterol ester because it has the same temperature and enthalpy as the smectic–liquid transition of cholesterol ester isolated from HDL, and the same temperature as the smectic–liquid transition of cholesterol ester observed by polarising microscopy of heat disrupted HDL. Further, a similar transition is not observed in any of the other lipid or protein components of HDL.

To determine if apo-A1 could be removed from HDL at 37 °C, we incubated intact HDL with turbid suspensions of a synthetic phospholipid, dimyristoyl lecithin (DML). There was clearing of phospholipid turbidity (measured by the decrease in optical density at 640 nm), indicating dissolution of multilamellar phospholipid liposomes. Differential scanning calorimetry tracings of incubated mixtures of HDL and DML are shown in Fig. 2. The addition of HDL to DML resulted in disappearance of the phospholipid pretransition, and broadening and decrease in enthalpy of the main transition. In mixtures containing an excess of HDL, there was complete clearing of turbidity and a single thermal transition with a peak temperature of 25.5 °C (Fig. 2*d*), identical to that observed in complexes of delipidated apo-A1 with DML (Fig. 2*e*). At higher ratios of DML to HDL, there was partial clearing of phospholipid turbidity and thermal transitions with double peaks were observed, the lower temperature component (23.5 °C) corresponding to the gel–liquid crystalline transition of DML multilamellar liposomes (Fig. 2*a*), and the higher temperature component (25.5 °C) to phospholipid–apoprotein complexes (Fig. 2*e*).

Phospholipid–apoprotein complexes, prepared from the delipidated apoproteins of HDL, are disks of phospholipid bilayer about 110×55 Å (ref. 15), which typically appear stacked on edge in negatively stained EM preparations[16]. Such structures were identified in all incubated mixtures of HDL and DML (arrow, Fig. 3*a*), and not in control preparations (Fig. 3*b*). The calorimetric and EM experiments indicate the presence in these mixtures of discoidal DML–apoprotein complexes containing a phospholipid bilayer. In optically clear mixtures (containing no uncomplexed phospholipid), more than 95% of spherical particles observed by EM were between 135 and 210 Å in diameter (for example mean diameter ± s.e.m. = 145 ± 5.1 Å ($n = 150$) in a mixture cointaining 50% DML/50% HDL apoprotein). Since more than 95% of the spherical particles observed were larger than those present in control HDL preparations (Fig. 3*b*), an increase in size of the HDL particle appears to accompany formation of phospholipid–apoprotein complexes. This finding was confirmed by recentrifugation at d 1.125 g ml^{-1} of HDL_3 (1.125–1.21 g ml^{-1}) previously incubated with DML. A major portion of the HDL_3 was found to float between 1.063 and 1.125 g ml^{-1} and compositional analysis showed this was not due to uptake of phospholipid by the HDL_3. The increased size and decreased density of HDL_3 resulting from incubation with DML is consistent with fusion of lipoprotein particles secondary to the removal of apoprotein. Further, the d 1.25 g ml^{-1} infranatant was found to be enriched in DML and apo-A1, indicating the presence of phospholipid–apo-A1 complexes.

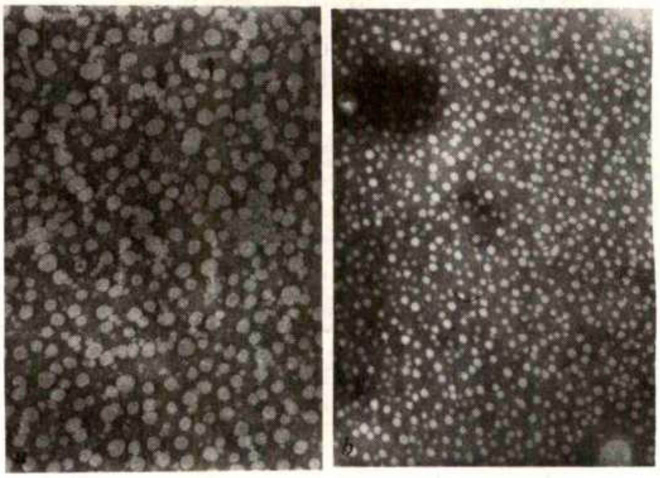
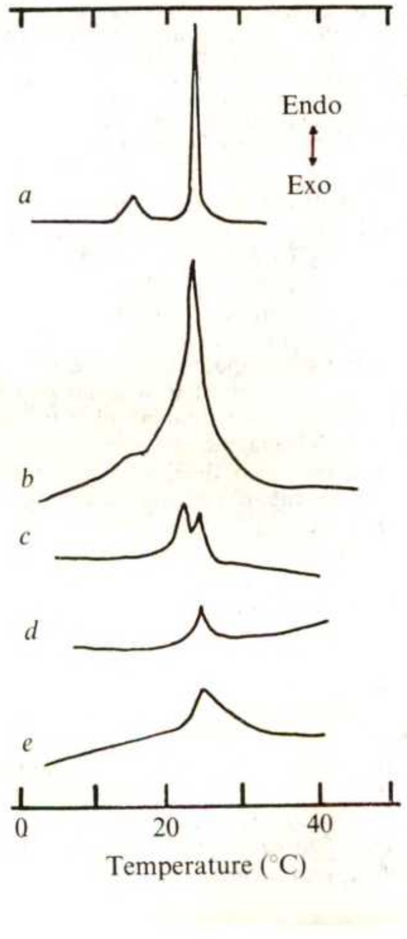


Fig. 3 Electron micrographs, negatively stained with Na phosphotungstate, *p*H 7.3. *a*, DML and HDL, (DML/HDL_3 apo-protein = 2/1). Disks stacked on edge are shown by the arrow. *b*, Control HDL_3 preparation. Magnification of original ×33,000.

Fig. 2 Differential scanning calorimetry tracings of incubated mixtures of DML and intact HDL: *a*, DML alone; *b*, DML and HDL (DML/HDL apoprotein = 12:1, w/w; *c*, DML and HDL (4:1); *d*, DML and HDL (1:1:2); *e*, DML and apo-Al (1:1.2). HDL (4–15 mg per ml protein) prepared by centrifugation was added without further concentration to unsonicated turbid suspensions of DML (about 4 mg ml^{-1}). DSC experiments were performed with 75 λ aliquots of mixtures of HDL and DML incubated under N_2 at 37 °C for 12 h, by which time all changes in turbidity were completed. The tracings were recorded at different sensitivities. Apo-Al and apo-Al–DML complexes were prepared as described previously[11].



The interaction of the apo-A1 of intact HDL with synthetic phospholipids suggests a possible mechanism for modification of cell membranes. A detergent-like solubilisation of areas of membrane determined by characteristics such as lipid fluidity and cholesterol content could allow an efficient and selective regulation of membrane properties.

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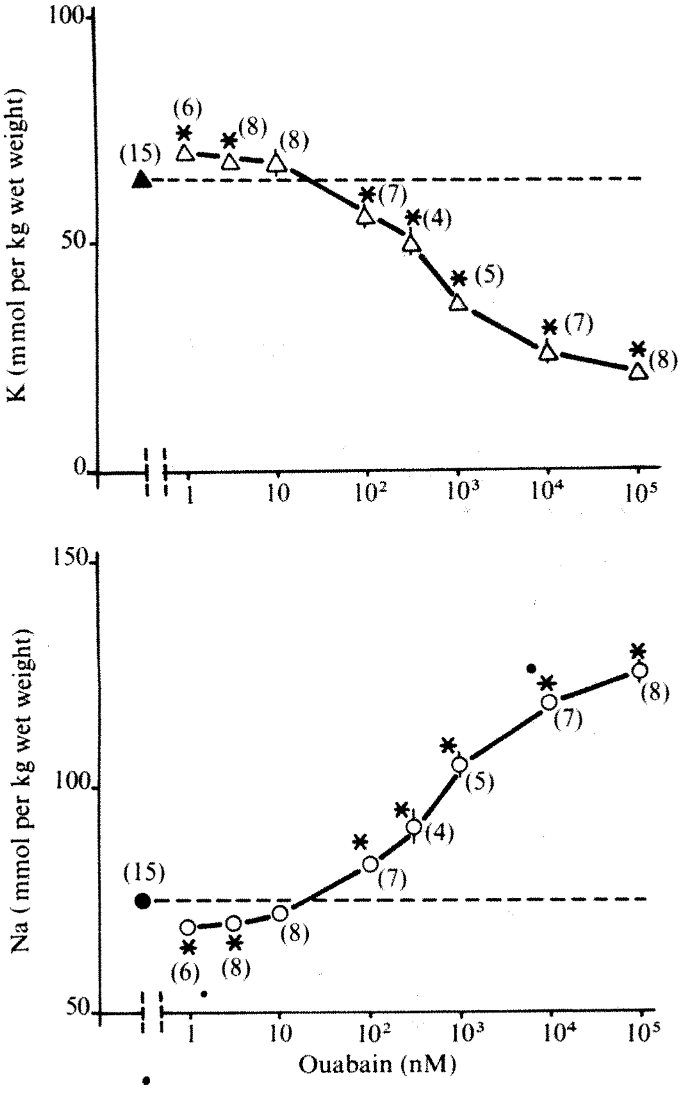
Binding sites related to ouabain-induced stimulation or inhibition of the sodium pump

THE concentration of free cardiac glycosides in the blood of patients treated for heart failure ranges between 1 and 5 nM (refs 1–3). Studies on human heart slices have shown that, within this range of concentrations, digitoxin does not inhibit the sodium pump but does stimulate ^{42}K uptake[3]. Similar observations have been made with isolated guinea pig atria: low concentrations of ouabain (near 1 nM) stimulate ^{42}K uptake whereas higher concentrations inhibit it[4]. These results are in agreement with recent electrophysiological observations on Purkinje fibres showing that low doses of ouabain produce changes in the K gradient that reflect stimulation of the sodium pump[5]. It has also been observed that the stimulation of the sodium pump by ouabain is associated with a positive ionotropic effect[6]. Earlier studies with guinea pig atria[4] and other preparations[7–9] showed that the binding of ouabain occurs on non-specific and specific sites. The specific binding is fitted by a Langmuir curve with an equilibrium constant close to that estimated by measuring the inhibition of the sodium pump. We report here the existence of a binding with a higher affinity associated with the stimulation of the sodium pump.

Fig. 1 Na and K content of stimulated left guinea pig atria incubated for 3 h at 30 °C in the presence of various concentrations of ouabain. Abscissa: ouabain concentration. Ordinate: ionic content determined, after mineralisation, by atomic absorption spectroscopy[11]. Each point represents the mean of a number of individual determinations given between brackets. The limits of s.e. of mean are reported when they exceed the diameter of the symbol. The asterisk indicates that the ionic content of treated atria was different from controls ($P < 0.02$). The dotted line indicates the mean content of controls (closed symbols).



Experiments were carried out with isolated left guinea pig atria bathed at 30 °C in physiological solution (mM: NaCl 137, KCl (unless otherwise stated) 6, $CaCl_2$ 1.82, $MgCl_2$ 0.105, $NaHCO_3$ 11.9, glucose 5.5) equilibrated with a mixture of 95% O_2 and 5% CO_2. The atria were electrically stimulated at a rate of 3.3 Hz (stimulus of 10 ms, strength at least twice threshold); their resting tension was ±500 mg.

Figure 1 shows the changes in K and Na contents measured after an incubation of 3 h in the presence of various concentrations of ouabain. At 1 nM and 3 nM ouabain, the Na content was lower and the K content was higher than in control preparations. Since the inulin diffusion space was not modified, these changes reflect a stimulation of the sodium pump. Higher concentrations of ouabain evoked a gain in Na content and a fall in K content, due to the well documented inhibition of the sodium pump[9,10].

The uptake of ouabain has been measured in the same experimental conditions. ^{3}H-ouabain was added together with ^{14}C-inulin in the incubation fluid. This procedure allowed us to correct tissue ouabain uptake for extracellular ouabain content. Cellular ouabain uptake showed both a linear and a saturable component, confirming earlier results obtained with ouabain concentrations ranging from 30 to 10^3 nM. This uptake was found to fit the equation[4]

$$U = aC_m + \frac{bC_m}{(C_m + K_b)} \qquad (1)$$

where U is the tissue concentration corrected for cardiac

Table 1 Ouabain content of guinea-pig atria corrected for cardiac glycoside in the inulin space

Ouabain medium concentration (nM)	Ouabain cellular content (nmol kg^{-1}) Observed	Calculated	P of the difference (observed – calculated)
a 6 nM KCl			
1	1.1±0.08 (8)	1.3	NS
1.5	2.6±0.2 (8)	1.9	$0.001 < P < 0.01$
3	5.8±0.5 (8)	3.8	$0.001 < P < 0.01$
6	10.4±1.0 (8)	7.6	$0.02 < P < 0.05$
8	10.6±0.67 (7)	10.0	NS
10	12.0±0.67 (8)	12.5	NS
30	37.9±5.1 (4)	36.2	—
10^2	112±23 (4)	108	—
10^3	592±48 (4)	587	—
10^4	3,550±126 (4)	3,550	—
b 2.7 mM KCl			
0.5	1.1±0.1 (8)	0.7	$0.02 < P < 0.05$
0.9	1.5±0.09 (7)	1.2	$0.01 < P < 0.02$
1	1.7±0.2 (8)	1.4	NS
3	4.7±0.8 (8)	4.1	NS
10	14.1±1.4 (8)	13.1	NS
30	35.5±2.8 (7)	36.3	—
10^2	96.6±4.0 (7)	96.6	—
10^3	389.5±13.3 (8)	390	—
10^4	2,279±94.7 (8)	2,279	—

Stimulated left guinea pig atria were incubated for 3 h in the presence of increasing concentrations of ouabain. The ^{14}C-inulin space was estimated for each atrium. The radioactivity was measured as reported before[11]. Values are presented as means ± s.e. of mean. The number of determinations is given between brackets. The incubation time of 3 h was sufficient for complete equilibration, except for ouabain 1 nM and 6 mM KCl. For the latter concentration, the equilibrium content reached after 4 h was equal to 2.1±0.09 nmol kg^{-1} ($n = 8$). The experimental observations are compared with values of U calculated from equation (1) using for the parameters the values estimated for ouabain concentrations between 3 and 10^3 nM. The latter were for 6 mM KCl: a (l per kg wet weight) 0.319, b (nmol per kg wet weight) 372, K_b (nM) 389; for 2.7 mM KCl: $a = 0.206$; $b = 219$; $K_b = 193$.

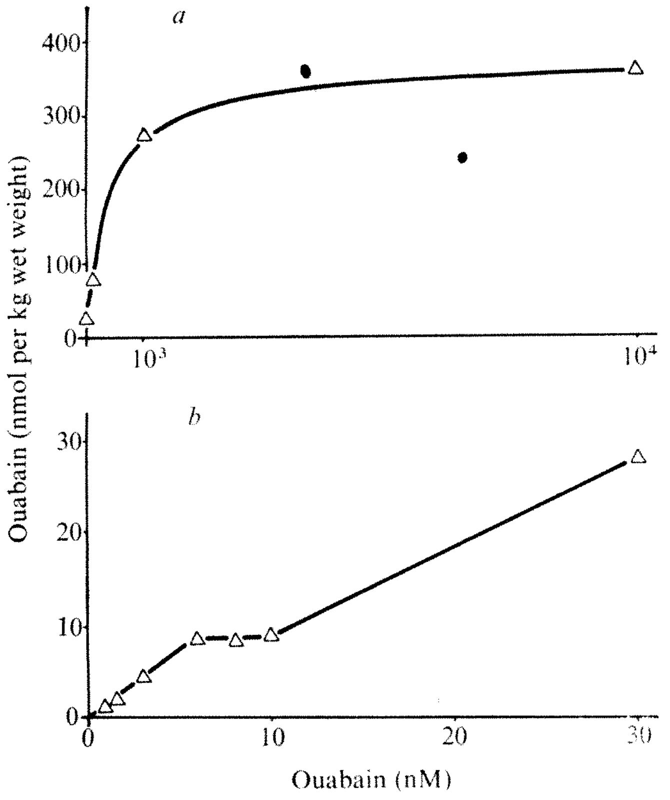


Fig. 2 Specific uptake of ouabain by guinea pig atria. Ordinate: tissue content of ouabain corrected for cardiac glycoside concentration in the inulin space and for the non-specific uptake aC_m. Abscissa: concentration of ouabain in the incubation fluid. Experiments were performed at 30 °C. The ^{14}C-inulin space was estimated for each preparation. The limits of s.e. of mean are shown when they exceed the diameter of the symbol. The number of determinations is given on the graph. Radioactivity was counted as reported before[4,11]. The lower concentrations in the range covered in *a*, are shown on an expanded scale in *b*.

glycoside content of inulin space, C_m is the cardiac glycoside concentration in the medium, a is the proportionality constant for the linear non-saturable uptake, b is the capacity and K_b is the equilibrium constant for the saturable binding sites. Values of the constants computed as described previously[4] are reported in Table 1*a*. The value of a, the non-saturable uptake constant has been estimated in two other experiments. By isotopic dilution, using a large excess of non-radioactive ouabain, constant a was 0.353 ± 0.0216 l kg^{-1} ($n = 12$). When ouabain uptake was measured in the presence of a large excess of digitoxin acting as a competitor[4] the nonspecific uptake of ouabain yielded a value of constant a equal to 0.358 ± 0.012 l kg^{-1} ($n = 4$).

The saturable ouabain uptake was obtained by subtracting the non-saturable uptake from the total uptake at ouabain concentrations ranging from 30 nM to 10^4 nM (Fig. 2*a*). The concentration of ouabain at which half of the saturable binding sites were occupied was equal to 389 nM, a value close to the concentration producing a 50% inhibition of the sodium pump (500 nM).

As shown in Table 1*a* for ouabain concentrations higher than 10 nM, there was a good agreement between experimental and computed ouabain tissue contents. At ouabain concentrations between 1 nM and 10 nM, ouabain tissue content was higher than that expected from equation (1) using the parameters estimated for ouabain concentrations between 30 nM and 10^3 nM.

Figure 2 illustrates the relation between ouabain concentration and specific ouabain binding. It shows two saturable components: one at concentrations ranging from 30 to 10^4 nM (Fig. 2*a*) and the other at ouabain concentrations between 1 and 30 nM (Fig. 2*b*). This indicates the existence of a high affinity and of a low affinity uptake process. The capacity of the high affinity process is only 3% of the capacity of the low affinity one. When the relation between specific binding and ouabain concentration was plotted according to Hill[10], the slope was equal to 2 for the high affinity binding whereas it was 1 for the low affinity binding. This analysis allowed to estimate that the concentration producing a 50% saturation of the high affinity binding was 2.5 nM.

In isolated mammalian cells[7] and in smooth muscle[8], extracellular potassium (K_o) competes for both the binding and the inhibitory effect of doses of ouabain higher than 10 nM. Changes in atria excitability, observed when K_o is lower than 2 mM and higher than 8 mM, allowed a limited study of the effect of K_o on high- and low-affinity binding (Table 1). In KCl 2.7 mM, the apparent equilibrium constant of the low-affinity binding was lower than in KCl 6 mM, and the high-affinity component of the binding was observed at lower ouabain concentrations. These observations suggest that the binding of ouabain to both high-affinity and low-affinity sites is to the sodium pump.

The present findings indicate that two populations of sodium pumping sites might coexist. Recent reports have shown that external Na inhibits the sodium pump by an allosteric mechanism[12,13]. It might be that the high- and low-affinity ouabain binding sites are different states of the same population of receptors. Further experiments are required in order to explore these possibilities.

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Clarification of role of ATP in red-cell morphology and function

RECENTLY there has been a tendency to ascribe many energy-dependent functions of other types of cells to red cells. The widespread belief that red cell form and deformability depend on cellular ATP levels and that membrane internalisation is an energy-dependent phenomenon are cases in point[1–4]. ATP has indeed been shown to control phagocytosis, pinocytosis and locomotion in white cells; the confusion over whether it is pinocytosis (which requires energy) or endovesicle formation (which does not) that occurs in erythrocytes may be due to the observation[5] of the former phenomenon in erythrocyte precursors (erythroblasts and reticulocytes). To establish that pinocytosis occurs also in the ultimate cell of the series—the erythrocyte—it is necessary to demonstrate that internalisation of the cell membrane is dependent on energy; and thus on temperature, *p*H, time, and ATP. This is not the case and results of the investigation described here show that ATP does not directly control either the form and deformability or membrane internalisation of red cells and that phenomena secondary to ATP depletion are responsible for the changes induced.

ATP depletion of red cells was accomplished by incubating washed red cells at 37 °C in either a glucose-free Tris-buffered saline medium or in the same medium containing 1 mM iodoacetate and 0.2% glucose. In both the systems the ATP content of the red cells at various periods of incubation was measured by luciferin–luciferase enzyme assay. The deformability of red cells was determined using the Ektacytometer[6]. Membrane internalisation was produced by treatment with chlorpromazine in the concentration range 0.3 to 0.5 mM, and internalisation of membrane demonstrated by morphological observations using phase contrast, transmission and scanning electron microscopy and by measurement of the deformability and osmotic fragility of cells.

Figure 1 shows the ATP content and form of human red cells as a function of incubating time in two media. In the glucose-free medium the discocyte–echinocyte transformation occurs by a progressive increase of echinocytes which are formed in the presence of discocytes, thus demonstrating heterogeneity of the morphology of the population before complete ATP depletion.

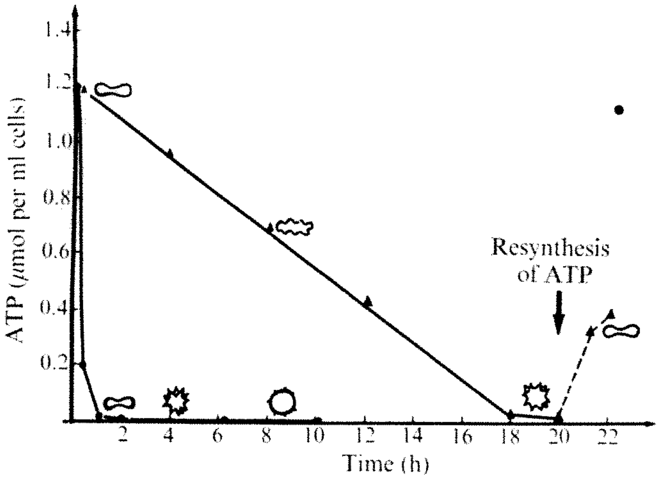

Fig. 1 Changes in ATP content and red-cell morphology with incubation time in different isotonic media: ●, changes obtained by incubation of cells in Tris-buffered saline containing 1 mM iodoacetate. ▲, Changes after incubation in Tris-buffered saline containing no glucose. All the incubations were carried out at an haematocrit of 5%. The *p*H was maintained at 7.4 and the temperature at 37 °C during the whole course of incubation.

When erythrocytes were incubated in a medium with iodoacetate it was possible to maintain discocytic red cells with zero ATP. By prolonging incubation time to 3 or 4 h, a progressive and complete discocyte–echinocyte transformation took place in the presence of iodoacetate. This shows that shape changes will ultimately, though not initially, be caused by effects secondary to ATP depletion that is; by alteration of the ionic equilibrium, loss of cations, and modification of the integrity of the membrane.

For the same cellular concentrations of ATP we can obtain red cells in various morphological states, suggesting strongly that the ATP concentration *per se* does not control cell shape and that ATP is only necessary for maintaining the integrity of other systems that are responsible for the discocytic form. Weed[7] has postulated that ATP-Ca^{2+} ratio is important in controlling form and deformability; and although we have not measured Ca^{2+} levels in the cells during these incubations, we have obtained identical results with incubating media containing chelating agents EDTA and EGTA which were used for the purpose of ruling out the possibility of external Ca^{2+} penetrating the cells during or after ATP depletion. Deformability data shown in Table 1 demonstrate that normal discocytes and discocytes with zero ATP have identical deformabilities. In both of the incubating media reduction in deformability could only be detected after the sphero-echinocyte stage has been reached. Measurements of red cells treated with lysolecithin permitted us to evaluate deformability changes in echinocytes with normal ATP levels. Here again, decrease in deformability could only be detected after the sphero-echinocyte stage had been reached.

Deformability of red cells measured at 4 °C and 37 °C was almost identical.

A dilute suspension (1%) of washed human red cells (discocytes) treated with 0.3 mM chlorpromazine underwent membrane internalisation and were transformed into spherocytes. These spherocytes with endovesicles (Fig. 2) were undeformable (Table 1) and had increased sensitivity to osmotic stress (50% haemolysis at 225 mOsm compared to 50% haemolysis at 140 mOsm for discocytes). When we treated discocytes having no ATP with 0.3 mM chlorpromazine we obtained exactly the same results. These spherocytes also had endovesicles (Fig. 2), reduced deformability and identical increased sensitivity to osmotic stress. Thus the absence of a role for ATP in membrane internalisation in red cells was clearly demonstrated.

The results presented here indicate that in our experimental conditions with intact red cells a direct role of ATP in red-cell membrane function could not be demonstrated. To demonstrate that membrane internalisation could be an energy-dependent phenomenon one should avoid inducing ATP depletion and at the same time a change of red cell shape. According to Deuticke a fixed concentration of chlorpromazine forming vacuoles in a discocyte would not be active on an echinocyte; that is, endocytosis would not

Table 1 Correlation between red-cell shape, ATP content and ability to deform in a free shear field

Red-cell shape	ATP content (μmol per ml RBC)	Average red-cell dimensions* No stress (μm)	At a shear stress of 750 dyne cm^{-2} (μm)
Discocytes	1.2	8.0	16.5
	0.0	8.0	16.5
Echinocytes III	1.2 LPC	5.5	16.0
	0.0	5.5	16.0
Sphero-echinocytes	1.2 LPC	5.5	8.0
	0.0	5.5	8.0
Sphero-stomatocytes with endovesicles	1.2	5.5	6.5
	0.0	5.5	6.5

Red-cells were suspended in a viscous dextran solution (0.14 Poise) and subjected to a shear stress of 750 dyne cm^{-2}. LPC = lysophosphatidylcholine-treated red cells.

*The average red-cell dimensions represent either the diameter of the disk or sphere when no stress is applied, or—when cells are deformed—the size of the larger axis of the ellipse obtained under stress.

occur in the latter cell. In fact we can show that echinocytes with normal or reduced ATP levels can be induced to form endovesicles at higher concentrations of chlorpromazine.

Another argument against energy requirement for membrane internalisation is that identical endovesicle formation was obtained at 4 °C and 37 °C. Neither was *p*H dependence found, in particular for low values compatible with complete solubility of the drug. A false *p*H dependence should result from precipitation of the drug at figures above 7.4.

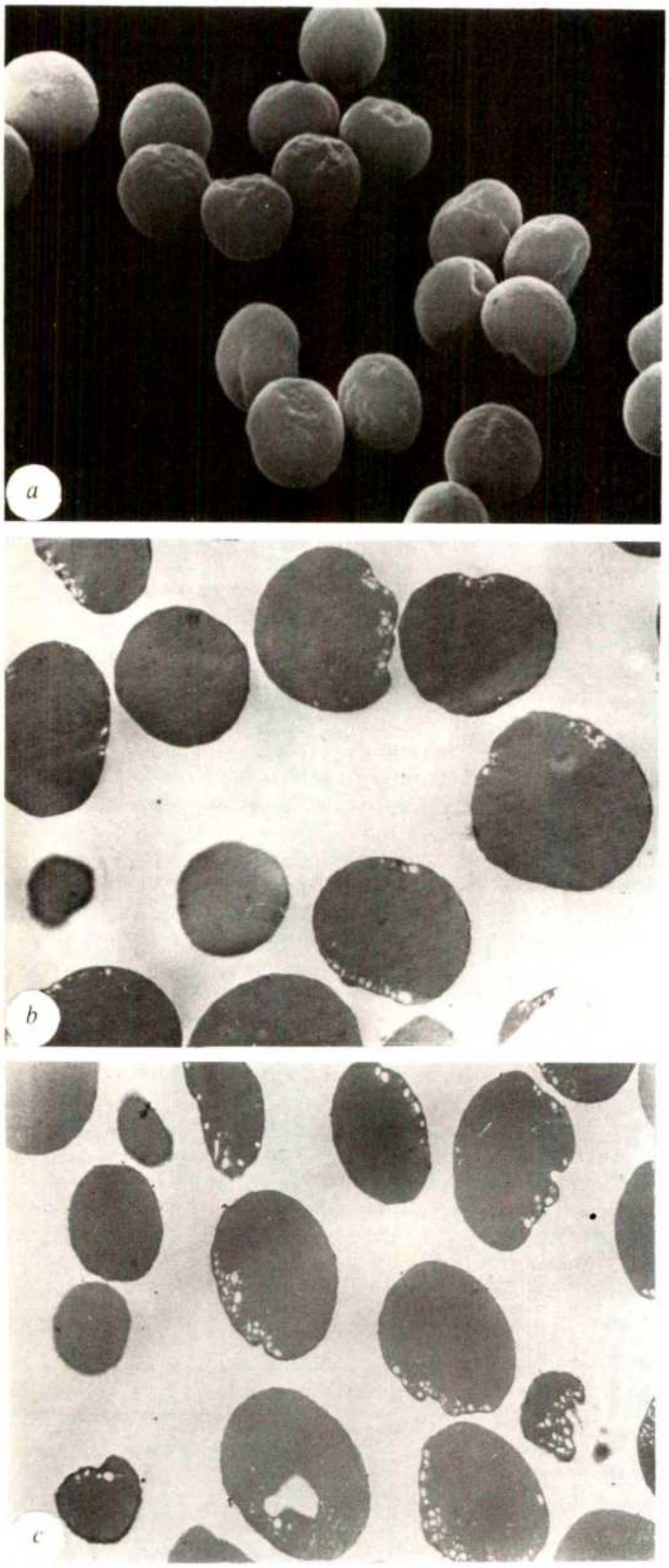

Fig. 2 *a*, Spherostomatocytes obtained by treating normal human discocytes with 0.3 mM chlorpromazine as seen after fixation in scanning electron microscopy. *b*, Endovesicles in spherostomatocytes after treatment with chlorpromazine of red cells containing normal ATP as seen in transmission electron microscopy. *c*, Endovesicles in spherostomatocytes containing no ATP. Red cells were incubated at 37 °C with 1 mM iodoacetate, then treated with chlorpromazine and observed using electron microscopy.

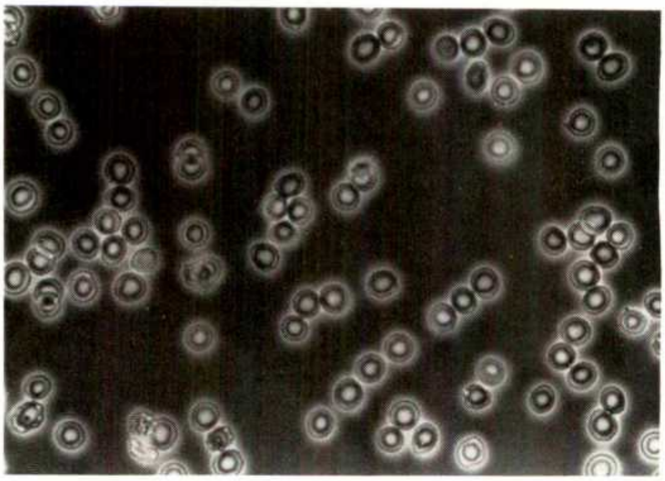

Fig. 3 Red cells incubated 1 h at 37 °C in a Tris-buffered medium containing 1 mM iodoacetate; ATP was < 0.05 μmol per ml red cells. Cells were fixed in 1% glutaraldehyde and observed with a phase contrast microscope (magnification × 290). 95% of the cells are discocytes, 5% are echinocytes 1 to 3.

A last point is the absence of time dependence since vacuoles are abundantly formed very rapidly (within seconds) as is shown by using high concentration of the chemical agents and rapid fixation of the red cells. During the same experiment no measurable ATP depletion could be detected.

Our results are difficult to compare with those using ghosts, as it can be considered that haemoglobin-free red cells represent a very different model.

In contrast, white cell deformability, phagocytic capacity and locomotion are all strong functions of temperature and are inhibited by depletion of cellular ATP levels. Our results, which conclusively show that red-cell form, deformability and membrane internalisation are not energy-dependent functions, taken together with those of others for white cells, strongly suggest that these two cells have different membrane systems for controlling these functions.

We think our data are most easily interpreted by a physicochemical hypothesis as proposed by Singer and Sheetz[8]. According to them, amphiphilic molecules are asymmetrically distributed in the membrane, that is preferentially in the interior part of it for cationic compounds, thus leading to shape changes and vacuole formation.

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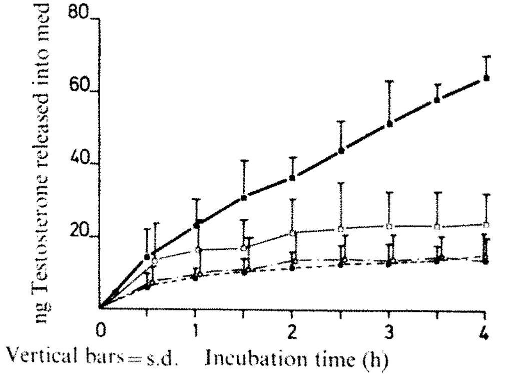
Do androgens increase Leydig cell sensitivity to luteinising hormone?

TARGET tissue responsiveness to hormones is not a static phenomenon, but is often influenced by the endocrine situation. For example, the sensitivity of the Leydig cell in the rat to luteinising hormone (LH) stimulation is not constant but gradually increases during prepubertal development[1]. Moreover, during treatment of hypogonadism in the human male, human chorionic gonadotrophin (hCG) therapy is often increasingly efficacious in stimulating testosterone secretion after repeated injections—a probable manifestation of induced target cell sensitivity. It is possible that androgen receptors in the Leydig cell[2,3] are involved in the regulation of these changes in sensitivity. They are specific for testosterone and dihydrotestosterone[4,5] and are distinct from the oestrogen receptors in the same cell[3,6]. We have investigated a possible role for these androgen receptors using the testicular feminised male (Tfm) rat. This rat has a male genotype and abdominal or inguinal testes, but a typical female phenotype because of a genetically determined lack of androgen receptors and a generalised target organ insensitivity to androgen[7]. We report here that the Leydig cells of the Tfm rat testis have much smaller number of LH/hCG receptors than normal, and this is associated with a low sensitivity to hCG.

To determine whether the responsiveness of Leydig cells of Tfm rats to hCG differed from that of normal males, we incubated Tfm and normal testes with the same dose of hCG. Figure 1 shows the response in terms of testosterone released into the medium. The Tfm testes exhibited a markedly impaired response, with treated testes releasing the same quantities of androgen as did control, unstimulated testes.

What defines the level at which a tissue will respond to a hormone stimulus? Most workers agree that the number of hormone receptors is one of the primary factors controlling the degree of responsiveness of a target tissue to a hormone. Therefore we investigated whether the Tfm testis had a normal content of LH receptors. Figure 2 shows Scatchard plots and saturation curves of LH binding to membrane receptors isolated from the testes of Tfm rats and their normal litter mates (NL). Although LH receptors in normal and Tfm testes had similar binding affinities, there were many fewer in the Tfm testes, which had approximately 20% of the number found in the normal situation when expressed in fmol per mg of protein and 5–10% when calculated per testis. As there seem to be more Leydig cells in the Tfm rat testis than in normal testicular tissue[8], these results suggest a considerable difference in the number of LH receptors per cell. The lack of response of the Tfm testis to hCG is probably due to the reduction in the number of LH receptors on the Leydig cell membranes. The fact that the Tfm syndrome is characterised by a reduced ability of androgens to exert a physiological and biochemical effect indicates that, in the normal situation, androgens are necessary for the normal induction of LH receptors in the Leydig cell. It is possible that this effect is mediated through the androgen receptor since the Tfm Leydig cells, contrary to the normal situation, have no androgen receptors[5].

Fig. 1 The effect of hCG on testosterone secretion from the testis of Tfm and normal litter mates (NL). Decapsulated testes from Tfm and NL rats ($n = 5$) were incubated with or without 3 ng of hCG in 2 ml of Eagle's tissue culture medium. One testis from each animal was incubated in the presence of hCG and the contralateral testis served as control (without hCG). Incubation was performed for 4 h at 34 °C in a Dubnoff shaker, with a gas phase of 95% O_2 and 5% CO_2. Aliquots (25 μl) were taken from media after various times and testosterone was determined by radioimmunoassay. Vertical bars = standard deviation. ■, NL testes with hCG; □, NL testes without hCG; ○, Tfm testes with hCG; ●, Tfm testes without hCG.



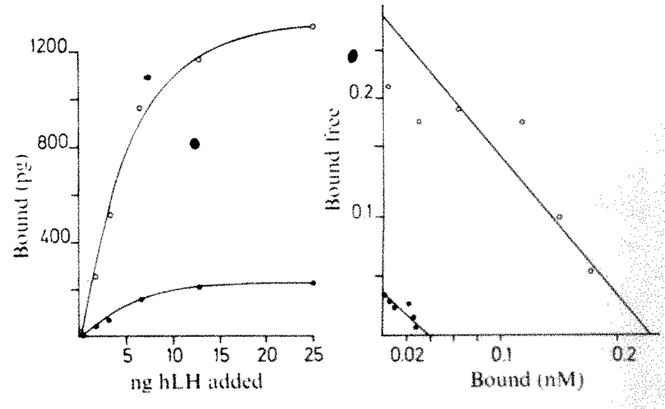


Fig. 2 Binding of human luteinising hormone (hLH) (15,000 IU per mg) to membrane receptors from the testes of Tfm (●) and NL (○) rats. Testes were homogenised in 10 volumes of phosphate-buffered saline (PBS), *p*H 7.4, and centrifuged at 27,000*g* for 30 min. The pellets were washed in the same buffer, recentrifuged and finally resuspended in 3 volumes of PBS containing 0.1% bovine serum albumin (BSA) and 0.1% Neomycin. Fifty μl (300 μg of protein) of membranes were equilibrated at 22 °C for 20 h with 10,000 c.p.m. of ^{125}I-hLH and various amounts of non-labelled hLH (0–20 ng) in a total volume of 200 μl. The pellets were centrifuged at 5,000*g* for 30 min, washed three times in the same buffer and the final pellets counted in a Packard Auto Gamma with 50% efficiency. Five μg of ovine LH (NIH–LH–S19) was used to assess nonspecific binding (6% of total binding). The data were plotted according to Scatchard (bound/free versus bound)[15] and the *x*-axis intercept gives the binding capacity.

Recent studies in our laboratory suggest that this induction takes place during a critical phase of prepubertal development. Treatment of immature male rats with a pure antiandrogen (cyproterone) results in a significant reduction in intratesticular and plasma testosterone without concomitant reduction in plasma gonadotrophin[9]. Similar treatment in the adult male stimulates testosterone secretion due to a central antiandrogen influence[10]. The fact that in the Tfm mouse the Leydig cells are arrested in their cytological development[7] strengthens the evidence for a short-loop positive influence of androgens on the Leydig cells during postnatal differentiation. The human Tfm syndrome, which is similar to that found in rats and mice, is associated with castration levels of LH[11] due to the impaired androgen feedback on the pituitary and hypothalamus. Plasma testosterone levels, however, are in the normal or subnormal male range[12,13], suggesting that Leydig cells in the human Tfm syndrome also show a reduced sensitivity to LH.

Study of the hormonal induction of peptide hormone receptors is the first step to an understanding of the mechanisms underlying fluctuations in tissue responsiveness. Androgens seem not only to induce LH receptors in the Leydig cell but also to stimulate the concentration of prolactin receptors in the rat prostate gland[13], indicating that the receptor-inducing action of the same steroid may be diverse and very complex.

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Role of prostaglandin endoperoxide PGG_2 in inflammatory processes

SINCE the discovery that non-steroidal anti-inflammatory agents (NSAI) are potent inhibitors of prostaglandin synthetase[1–3], there has been controversy about the role of primary prostaglandins (PGEs+PGFs=PGs) in inflammatory diseases. A parallel has been demonstrated between the ability of drugs such as indomethacin and aspirin to inhibit cyclo-oxygenase, the initial step leading to the formation of PGs, and anti-inflammatory activity[4–6]. The failure of administered PGs to fully mimic inflammation processes, however, is not consistent with this concept[7,8], and further studies have shown that other products derived from the reaction of cyclo-oxygenase with arachidonic acid (PGG_2, PGH_2 and thromboxane A_2) although quite labile, have biological activities exceeding the potency of the PGs themselves[9,10]. Since these ephemeral mediators are also subject to regulation by NSAI they too must be considered as potential causal agents of inflammation. We have investigated this possibility by examining the action of several NSAI on the total pattern of arachidonic acid oxygenation products. The results indicate that the endoperoxide PGG_2 has a pivotal role in acute inflammation.

During a search for potential non-steroidal anti-inflammatory drugs, one was found to possess a high degree of biological activity, yet it was inactive as an inhibitor in the routine PG synthetase assay[4]. In rat foot oedema, this compound, 2-aminomethyl-4-*t*-butyl-6-iodophenol (MK-447), exhibited good anti-inflammatory activity. Figure 1 shows the dose–response line for oral dosage compared with those for indomethacin and phenylbutazone. The ED_{50} values for indomethacin, MK-447 and phenylbutazone were 2.73, 17.3 and 27.7 mg kg^{-1} respectively. At the level of 50% inhibition of the foot oedema therefore MK-447 was approximately one-sixth as potent as indomethacin, and 1.6 times as potent as phenylbutazone. A similar high degree of activity in suppressing the swelling of the mouse's ear induced by croton oil[11] was also noted. Prostaglandin synthetase inhibitors are efficacious in both assays. It is interesting that the dose–response slopes for MK-447 in both the foot oedema and mouse ear assay, 24.5 and 47.0 respectively, differ from those of the two PG synthetase inhibitors, indomethacin (38.0 and 65.0) and phenylbutazone (38.5 and 65.0). This difference suggested a mode of action for MK-447 not involving inhibition of PG synthetase.

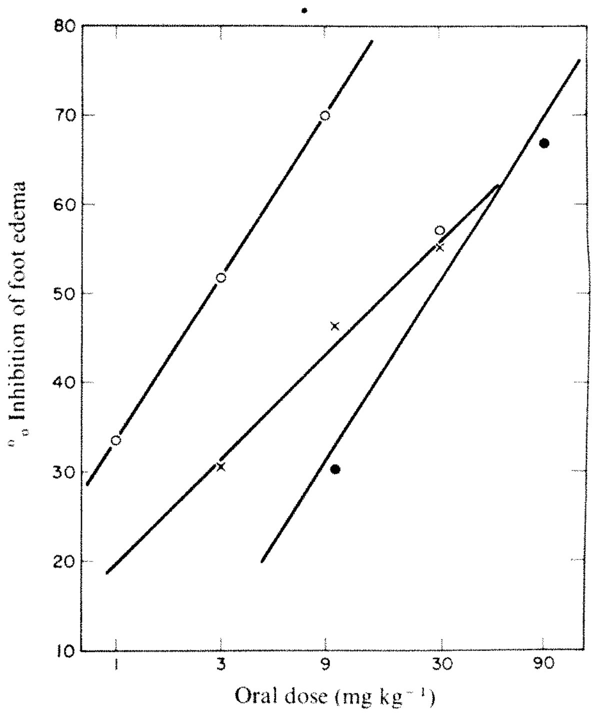

Fig. 1 Comparison of the anti-inflammatory activity of MK-447(×) with the PG synthetase inhibitors indomethacin (○) and phenylbutazone (●) on carrageenan-induced oedema in the rat foot. For indomethacin $y = 38.04 \log x + 33.43$ ($n = 780$ rats;) MK-447, $y = 24.5 \log x + 19.6$ ($n = 96$ rats); phenylbutazone, $y = 38.54 \log x - 5.61$ ($n = 306$ rats). Male Sprague–Dawley rats (150–170 g body weight) were used in groups of 6 each. Compounds to be tested were given by stomach tube in a water solution of 0.5% Methocel used as a suspending agent; 3 ml were given per rat. One hour later, 0.1 ml of 1% carrageenan suspension (Viscarin, Marine Colloids) was injected subcutaneously under the plantar surface of the right hind foot. The volume of the foot was determined immediately by immersion of the foot into mercury up to a previously marked ink spot; the volume of mercury displaced was recorded automatically. The mark was placed on the skin over the lateral malleolus. Three hours later, the volume was measured again; the difference between the two volumes was the swelling. Drug effects were calculated as percentage inhibition of the swelling, taking the swelling of the control group as 100%.

As noted, MK-447 did not inhibit the synthesis of PGs from arachidonic acid when a ram seminal vesicle preparation was used, nor did it inhibit the reaction when oxygen uptake was monitored. In fact, in the absence of cofactors, MK-447 stimulated overall prostaglandin synthesis in a microsomal preparation derived from ram seminal vesicles (Table 1). A similar ability to stimulate the synthesis of PGs was observed in incubated intact mouse ovaries and rat kidney slices (data not shown). These observations indicating that both inhibitors and stimulators of the formation of prostaglandins can suppress inflammatory responses can be interpreted to mean that an intermediate in their formation, apart from the prostaglandins, has a causal role in acute inflammation. The formation of such an intermediate would be expected to be blocked by PG synthetase inhibitors which act at the initial step in the sequence. Levels of such an intermediate would be depressed by the MK-447-facilitated conversion to PGs. In the events leading to the formation of

Table 1 Effects of non-steroidal anti-inflammatory agents on endoperoxides and PGs derived from enzymatic oxygenation of arachidonic acid

Product	Incubation time (min)	nmol per mg protein No additions	MK-447 (100 μM)	Indomethacin (10 μM)	Phenylbutazone (100 μM)	Aspirin (100 μM)
AA	0.5	23	10	43	24	44
PGG_2	0.5	16	6	1	0	0
PGH_2	0.5	4	27	0	20	0
AA	5	7	7	44	22	44
PGG_2	5	13	0	0	4	0
PGH_2	5	5	18	0	11	0
PGs	5	8	15	0	7	0

Values in each category represent a minimum of five experiments. Total radioactivity recovered was greater than 75% in each case and reproducibility of the values for each individual experiment was ±6%. Microsomes were prepared from ram seminal vesicles as described before[14]. The compounds were preincubated with 500–600 μg of microsomal protein in 0.2 ml of 0.1 M potassium phosphate buffer, *p*H 7.5, for 5 min at 25 °C. The reactions were initiated by the addition of labelled arachidonic acid (22 nmol containing 0.25 μCi of ^{14}C-1-arachidonic acid) and incubated for the times indicated. They were terminated by adding 0.05 ml of 0.3 M citric acid, and the products and remaining arachidonic acid were extracted into ether precooled to −70 °C. The entire procedure was carried out in the cold. Ether extracts were chromatogrammed at −20 °C on Whatman SG-81 silica-impregnated paper using ether–hexane–acetic acid (85:15:0.1) as described[24]. Incubation for 0.5 min resulted in the formation of only trace amounts of PGE_2 and $PGF_{2\alpha}$.

PGs from arachidonic acid (AA), that is $AA \rightarrow PGG_2 \rightarrow PGH_2 \rightarrow PGs$, either PGG_2 or PGH_2 would qualify as such an inflammatory agent. The overall sequence of these reactions is depicted in Fig. 2.

As Table 1 shows, approximately half the arachidonic acid is utilised by the microsomal enzyme leading to the formation chiefly of PGG_2 during a 0.5-min incubation. The formation of both endoperoxides is almost completely suppressed by indomethacin. This finding is consistent with an inhibitory effect on the cyclo-oxygenase step[12], the initial reaction in the oxidation of arachidonic acid by PG synthetase. Phenylbutazone inhibits the cyclo-oxygenase reaction less effectively, but significantly alters the ratio of endoperoxides in favour of PGH_2. Aspirin, at relatively high levels, blocks the formation of PG intermediates, a result consistent with an action to acetylate the cyclo-oxygenase[13]. Finally, MK-447 facilitates the enzymatic oxidation of AA to the endoperoxide PGH_2 in 0.5 min and ultimately to prostaglandins as reflected by the value obtained with the longer incubation.

The data presented here show that the quantitative effects of the NSAI studied differ markedly. But these compounds consistently and considerably depress levels of PGG_2, in spite of various effects on the other parameters even including a stimulation of the formation of PGs by MK-447.

Studies of several analogues of MK-447 (data not shown) revealed an obligatory requirement for the free phenolic group for both enzymatic and biological activity; the methyl ether was completely inactive in both parameters. Since phenol itself has been shown to stimulate both the uptake of oxygen by the cyclo-oxygenase and the overall synthesis of prostaglandins[12], a mechanism of action common to both MK-447 and phenol is suggested.

The capacity of phenol to stimulate the synthesis of prostaglandins has been reported to be linked to its ability to facilitate the conversion of the endoperoxide PGG_2 to

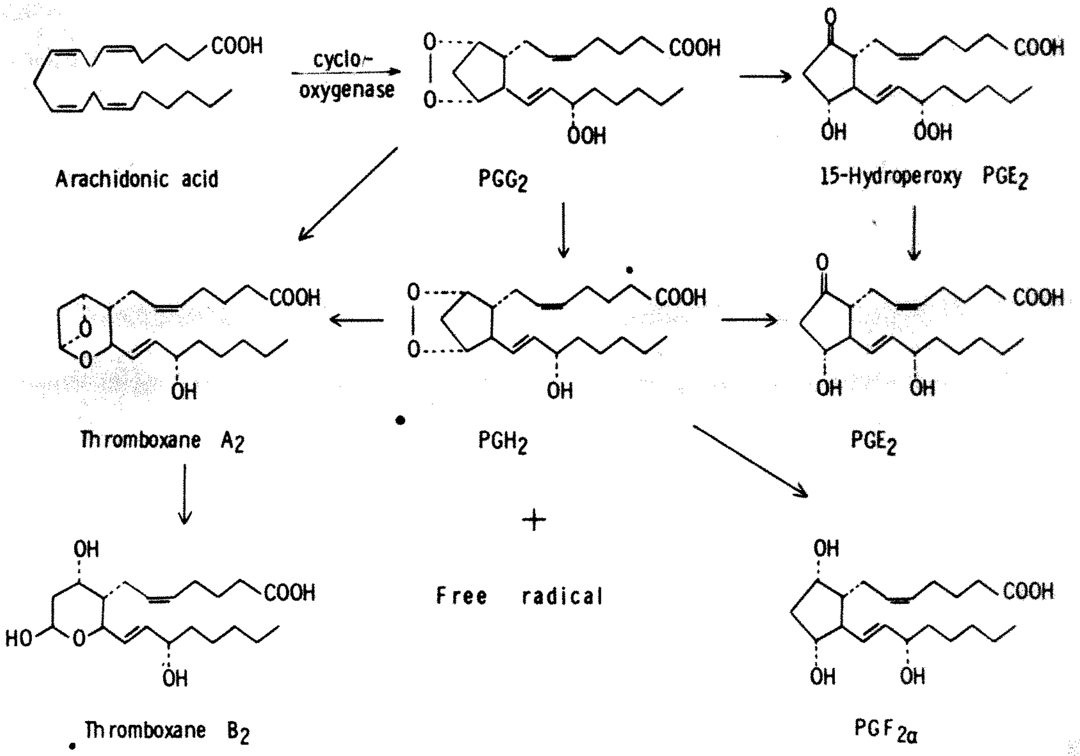

Fig. 2 Enzymatic conversion of arachidonic acid to potential mediators of inflammation.

Table 2 Anti-inflammatory activity of phenol in the mouse ear assay

Phenol (mg)	PMA (mg)	Phenol application relative to PMA	Average change (mg)*	% Inhibition	P
0.25	0	—	0.15 ± 0.07	—	—
0.5	0	—	0.13 ± 0.38	—	—
1.0	0	—	1.02 ± 0.39	—	—
0	0.05	—	14.8 ± 1.3	—	—
0.33	0.05	15 min pre.	11.0 ± 1.0	25.7	<0.05
0.33	0.05	with PMA	10.4 ± 0.71	29.7	<0.01
0.33	0.05	1 h post	10.8 ± 1.73	27.0	<0.05
0.33	0.05	2 h post	12.6 ± 1.3	14.9	NS
1.0	0.05	15 min pre.	6.9 ± 0.68	53.4	<0.01
1.0	0.05	with PMA	8.4 ± 1.54	43.2	<0.01
1.0	0.05	1 h post	10.2 ± 2.4	31.1	<0.10
1.0	0.05	2 h post	12.7 ± 2.0	14.2	NS

The mouse ear oedema assay[11] was used for these studies, but modified in the use of phorbol 12-myristate-13-acetate (PMA) in place of croton oil as the challenging agent. Groups of 6 mice were used for measurements of the action of phenol and PMA alone (each in 0.1 ml of acetone) and in combination. At times indicated after this challenge a second group received the acetone solution of phenol. Four hours later the mice were killed by cervical dislocation, 8-mm circles of ear were punched out, maintained in standard conditions of humidity and weighed.

*Values represent the increase in weight of the treated compared with untreated ear.

PGH_2 (ref. 14). The corresponding methyl ether is inactive. This effect on the endoperoxide interconversion is a property common to both phenol and methional, a well recognised radical scavenger. Electron paramagnetic resonance studies showed that phenol acts as a free radical scavenger in this reaction, causing stimulation of overall PG synthesis by sequestering the destructive oxygen-centred radical released in the conversion of PGG_2 to PGH_2 (ref. 14). Removal of this radical by phenol not only facilitates the conversion of PGG_2 to PGH_2 but also increases the net turnover of arachidonic acid by minimising oxidative inactivation of cyclo-oxygenase by this same destructive free radical.

Although phenol is inherently quite corrosive to tissues it has been used topically as a pain killer at low doses[15]. We do not know, however, of any report that phenol represses the oedema involved with inflammatory processes. Because of the property common to MK-447 and phenol in stimulating the conversion of PGG_2 to PGH_2 it was interesting to investigate whether phenol itself, like MK-447, had anti-inflammatory activity as measured in one of the *in vivo* test models of inflammation. We used the mouse ear oedema assay[11], but modified it by using phorbol myristate acetate (PMA), a component of croton oil, as the challenging agent. At levels up to 1 mg per site, applied topically, phenol had no significant effect on ear weight by itself when measured 4 h after application (Table 2). At this level a 53.4% reduction in tissue weight occurred when phenol was applied 15 min before the PMA challenge. Significant inhibition was also noted when phenol was applied 1 h after treatment with PMA and read at 4 h. Thus the ability of phenol to mimic the anti-inflammatory properties of MK-447 and their common actions to stimulate the conversion of PGG_2 to PGH_2 and overall PG synthesis are supportive of the concept that the mechanism of action of MK-447 relates to its ability to scavenge free radicals.

The most salient feature of these studies on the oxygenation of arachidonic acid is that levels of the endoperoxide intermediate, PGG_2, alone are depressed by all drugs examined. These data imply that PGG_2 has a pivotal role in acute inflammation. A particularly cogent finding is that one of these drugs, MK-447, can not only cause a depression of PGG_2 in an *in vitro* enzyme system, but also elicit an increased synthesis of PGEs by both microsomal enzyme and by incubated tissues. Thus the demonstrated anti-inflammatory activity of this compound in animal models of acute inflammation, coupled with its ability to stimulate the synthesis of PGs, is not compatible with the concept that primary prostaglandins (PGEs and PGFs) play a key role in inducing the symptoms of inflammation.

Since vasodilation, one component of acute inflammation, is well duplicated by administration of PGE_1 alone[16,17] it is reasonable to suggest that this aspect of the disease relates to the formation of PGEs. But the failure of administered PGEs to fully mimic the oedema[18,19] and pain[20] of inflammation points to PGG_2 as a prime factor in these two components of acute inflammation in view of the data presented here. In focusing on the endoperoxide it is necessary to consider that PGG_2 itself may not be the actual triggering agent in acute inflammation, but merely the precursor of such a substance. Such potential causal agents are shown schematically in Fig. 2. Thromboxane A_2, recently identified as the potent rabbit-aorta-contracting substance, is formed from PGG_2, and its derivation from this endoperoxide may be the preferred pathway[10]. Alternatively, the free radical formed in the metabolism of PGG_2 and tentatively identified as a hydroxy radical[14] may be the inflammatory component liberated in the enzymatic oxidation of arachidonic acid. Such hydroxy radicals have been reported to be very destructive to biological systems and their actions have been linked to inflammatory processes[21,22]. Evidence that peroxy acids are extremely effective pain-inducers[20], and that increased lipid peroxidation accompanies release of lysosomal enzymes[23] adds further credence to the concept that the hydroxy radical may be the relevant oxygenation product of AA in inflammatory diseases. Finally, it is necessary to consider hydroperoxy PGE_2 which also derives from PGG_2. Like PGG_2, 15-hydroxyperoxy PGE_2 is potentially able to liberate an oxygen-centred free radical. In any case we conclude from these studies that the primary PGs as represented by PGE_2 and $PGF_{2}\alpha$ are not the important inflammation-inducing agents obtained from arachidonic acid. Rather the endoperoxide, PGG_2, itself or a non-prostaglandin product(s) derived from it seems to be the major inflammatory agent formed during the enzymatic oxidation of arachidonic acid and blocked by the action of PG synthetase inhibitors, resulting in improvement of the symptoms of inflammation.

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Regulation of autosensitisation to encephalitogenic myelin basic protein by macrophage-associated and soluble antigen

AUTOIMMUNE diseases are characterised by an attack of an individual's lymphocytes against his own body's components, self-antigens. The immunogenicity of self-antigens can be defined operationally by their capacity to trigger lymphocytes bearing specific receptors. We have designed experiments to learn how a defined self-antigen can function as an immunogen *in vitro* and how the form of its presentation to self-recognising lymphocytes may abrogate this immunogenicity. We have found that myelin basic protein (BP) behaves as a self-immunogen when presented to lymphocytes by way of syngeneic macrophages. Moreover, recognition of this self-immunogen can be inhibited by the presence of the same self-antigen in soluble form during the primary lymphocyte–macrophage interaction.

BP is a self-antigen whose amino acid sequence and encephalitogenic determinants have been characterised[1]. Injection of BP in a suitable adjuvant produces in susceptible animals an autoimmune disease called experimental allergic encephalomyelitis (EAE) (ref. 2). The disease is manifested by histological evidence of lymphocytic infiltration of the central nervous system and is expressed clinically by paralysis. For induction of EAE, BP is usually injected in complete Freund's adjuvant; when the same self-antigen is administered in a soluble form without an adjuvant, it is not encephalitogenic. Moreover, under certain circumstances, treatment with soluble BP or its chemical analogues may suppress EAE (refs 3–5). Thus, the development of EAE seems to require suitable presentation of BP to the immune system.

To analyse the immunogenicity of BP, we focused on the initial phase of lymphocyte recognition of BP and separated this process from the subsequent differentiation of effector lymphocytes. We extended a previous observation that recruitment of immunospecific recipient lymphocytes can serve as a means of detecting whether recognition of self-antigens by initiator lymphocytes has occurred *in vitro*[6]. Thus, the recognition of BP was detected by testing the ability of the initiator lymphocytes to specifically recruit lymphocytes in highly inbred strain 13 guinea pigs. A three-phase system was developed (Fig. 1). In stage I monolayers of macrophages were prepared and pulsed with BP antigen. In stage II, the *in vitro* sensitisation phase, initiator lymphocytes were exposed to the BP-pulsed macrophages in the presence or absence of soluble BP. The initiator

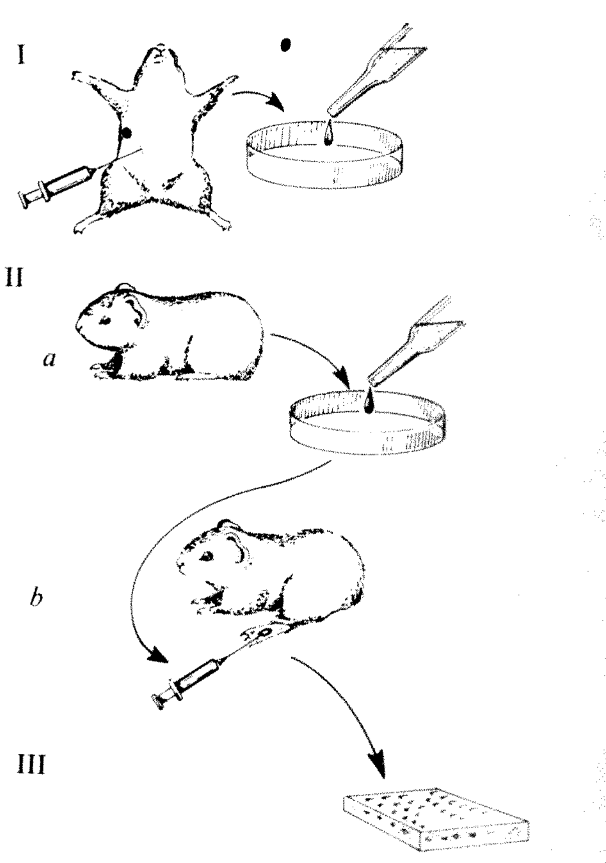


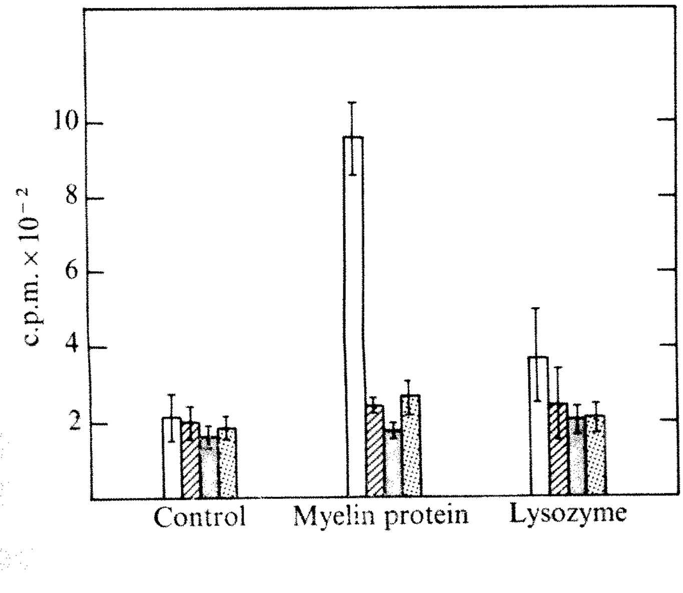
Fig. 1 Experimental format for study of the regulation of autosensitisation to BP. I, BP-pulsed macrophages. Peritoneal exudate macrophages were prepared from unprimed Strain 13 guinea pigs, 4 d after intraperitoneal injection of 15 ml sterile Drakeol, by lavaging the peritoneum with sterile phosphate-buffered saline (PBS) plus heparin, 5 units ml^{-1}. All procedures were then performed in RPMI + 25 mM HEPES buffer without serum at 37 °C; 20 × 10^6 macrophages were allowed to adhere to 100-mm Petri dishes for 2 h, then washed to remove non-adherent cells. The macrophages were then pulsed for one hour with 100 μg ml^{-1} of either BP or G,T. Antigen not associated with macrophages was removed by changing the medium three times. II, Sensitisation of initiator lymphocytes. *a*, 100 × 10^6 unprimed spleen cells were added to the macrophage monolayers. Some groups received soluble BP at a concentration of 100 μg ml^{-1} during lymphocyte–macrophage interaction. After 16 h of incubation of the lymphocytes on the macrophage monolayers, non-adherent cells were removed, then replated for 1 h to re-adhere detached macrophages. No more than 2.5% of the recovered cells seemed to be macrophages and more than 95% were small lymphocytes. The initiator lymphocytes were irradiated with 1,000 R to prevent their replication but not their ability to recruit *in vivo*[6]. *b*, Lymphocytes were then injected into the footpads of syngeneic animals at a concentration of 10 × 10^6 cells in 0.1 ml PBS. Some animals received injections of 10^6 antigen-pulsed macrophages in 0.1 ml PBS into the footpads. The macrophages were obtained from the plates by scraping with a rubber policeperson. Each group contained three or four guinea pigs. III, Assay of proliferative response of recruited lymphocytes. After 13 d the draining popliteal nodes were excised and lymph node cell suspensions of each group were pooled. After 1 h of adherence on plastic Petri dishes to remove debris and dead cells, 5 × 10^5 cells were placed in culture on Greiner microtitre plates. Medium for the assay phase consisted of RPMI + 25 mM HEPES + 1.25% foetal calf serum. 5 × 10^4 unprimed, unstimulated macrophages were irradiated with 2,000 R and added to each culture. Cultures were incubated with 15 μg ml^{-1} of various test antigens for 72 h, then 1 μCi of ^{3}H-thymidine was added for 16 h. Cultures were then collected on a MASH 2 cell harvester and counted in scintillation fluid in a Packard Beta counter. Results are reported as the mean and standard error of the four or five culture wells which comprised each group.

lymphocytes were then collected, irradiated to prevent their proliferation, and injected into syngeneic recipient guinea pigs. In stage III, the assay phase, the DNA proliferative response of recruited lymphocytes to BP served to indicate whether autosensitisation of the initiator lymphocytes had occurred.

The results in Fig. 2 demonstrate that BP was recognised, and triggered syngeneic spleen lymphocytes when presented on macrophages. Initiator lymphocytes were exposed to BP-pulsed macrophages and the lymphocytes were injected into recipient animals. Thirteen days later, recruited lymphocytes from the draining popliteal nodes of the recipient animals reacted to BP with a stimulation index (SI) of 4.4 (Fig. 2). The recruited lymphocytes did not respond to lysozyme, a protein of similar molecular weight and charge. Furthermore, lymphocytes incubated on macrophages not pulsed with antigen failed to recruit lymphocytes responsive to either BP or lysozyme, excluding a potential nonspecific stimulation resulting from the lymphocyte–macrophage interaction.

A control was designed to test whether passive transfer of BP, rather than *in vitro* sensitisation of the initiator lymphocytes could account for recruitment of recipient lymphocytes. Guinea pigs were injected with 500 μg of BP, a dose equivalent to the entire amount present in the *in vitro* culture. The recipient animals showed no evidence of sensitisation to BP (Fig. 2), suggesting that mere passive transfer of antigen could not have triggered active sensitisation to BP *in vivo*. Another control was done to test the possibility that antigen-bearing macrophages that had contaminated the injected lymphocytes acted as the initiators of recruitment. Recipients were injected with 1×10^6 macrophages that had been incubated with BP. This number of macrophages was at least 4 times greater than the number of macrophages actually observed to contaminate the injected lymphocytes. The response to this dose of macrophages was also insignificant (Fig. 2). In other experiments (unpublished) we found that injection of 5×10^6 or more BP-pulsed macrophages was needed to sensitise guinea pigs against BP *in vivo*. Thus, autosensitised initiator lymphocytes and not passively transferred antigen nor antigen associated with macrophages induced the recruitment of recipient lymphocytes against BP in these experiments.

Fig. 2 Immunogenicity of BP on syngeneic macrophages. Incorporation of ^{3}H-thymidine was measured in recruited lymphocytes from the draining popliteal lymph nodes of recipients, 13 d after injection. Recipients received initiator lymphocytes incubated on BP-pulsed macrophages (clear bars) or on macrophages with no antigen (diagonally hatched bars). Other recipients received 10^6 BP-pulsed macrophages (fine stippled bars) or 500 µg of BP directly (coarsely stippled bars). The groups of recruited lymphocytes were incubated without added antigens (control) or with 15 µg ml^{-1} of myelin protein (BP) or lysozyme.



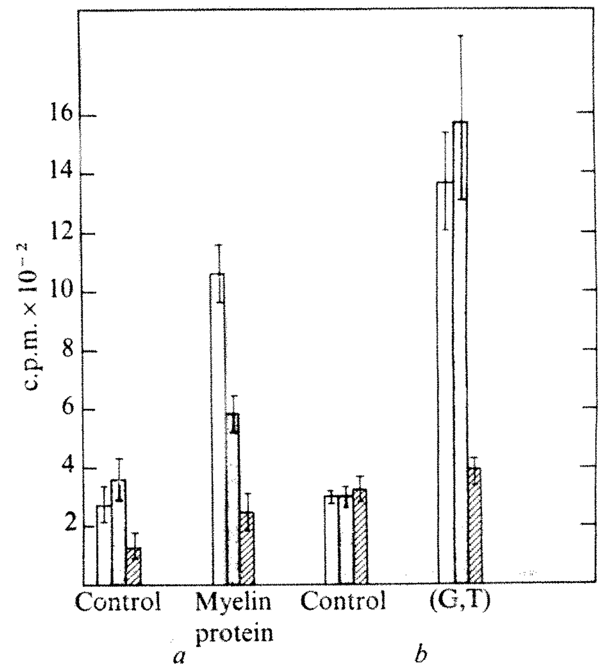


Fig. 3 Blocking of autosensitisation to BP by the presence of soluble BP. Incorporation of ^{3}H-thymidine was measured in recruited lymphocytes from the draining popliteal lymph nodes of recipients 13 d after injection. Recipient animals (Group *a*) received 10×10^6 initiator lymphocytes that had been incubated on BP-pulsed macrophages without (clear bars) or with 100 µg ml^{-1} BP in the culture (stippled bars). Other recipients (diagonally hatched bars) received 10^6 BP-pulsed macrophages. In Group *b* recipients received either 10×10^6 initiator lymphocytes incubated on G,T-pulsed macrophages without (clear bars) or with 100 µg ml^{-1} in the culture (stippled bars). Some recipients received 10^6 G,T-pulsed macrophages directly (diagonally hatched bars). The groups of recruited lymphocytes were incubated without added antigens (control) or with 15 µg ml^{-1} of myelin protein (BP) or G,T.

Next, we tested whether soluble BP might inhibit the activation of self-recognising initiator lymphocytes by macrophage-bound BP (Fig. 3). Initiator lymphocytes exposed to BP-pulsed macrophages recruited recipient lymphocytes to respond to BP (SI=3.9). However, when initiator lymphocytes were exposed to BP-pulsed macrophages in the presence of 100 μg ml^{-1} of BP in the culture medium, the response of the recruited lymphocytes was markedly reduced (SI=1.6; $P<0.001$). As above, injection of BP-pulsed macrophages (10^6) by themselves failed to recruit a response to BP (SI=1.8).

The inhibitory effect of soluble BP was found to be specific (Fig. 3). Initiator lymphocytes incubated on syngeneic macrophages pulsed with a copolymer of glutamic acid and tyrosine (G,T), recruited a response to G,T (SI=4.7). The presence of 100 μg ml^{-1} of soluble BP did not inhibit this response to G,T (SI=5.4), indicating that the soluble BP was not toxic to lymphocytes. The control injection of G,T-pulsed macrophages did not recruit a response to G,T (SI=12). Thus, the presence of soluble BP specifically blocked the induction of sensitisation to macrophage-bound BP and not to an unrelated antigen.

We have demonstrated that healthy guinea pigs possess lymphocytes that can recognise and be activated by a normal self-constituent, BP, when it is associated with syngeneic macrophages *in vitro*. These lymphocytes, which at least when *in vitro* possess receptors for guinea pig BP, can recruit immunospecific autosensitised lymphocytes in a syngeneic recipient. Indeed, normal as well as immune lymphocytes were observed to bind myelin basic protein, presumably by way of specific receptors[7].

In the experiments reported here we did not observe clinical or pathological signs of EEA in guinea pigs receiving 10^7 initiator lymphocytes. In earlier studies in this laboratory[8], lesions of EAE were produced in rats by the injection of about 4×10^8 initiator lymphocytes of thymic origin sensitised *in vitro* against a crude extract of rat brain. Thus, the development of overt EAE might require injection of many more than the 10^7 initiator lymphocytes sufficient to recruit a detectable proliferative response to BP. Although the conditions for induction of disease were not met, our results indicate that receptor-bearing lymphocytes can be induced to immune differentiation by BP associated with syngeneic macrophages. Furthermore, the presence of the same BP in soluble form can block the activation of lymphocytes by immunogenic BP.

These findings suggest that the mechanism involved in the encephalitogenic activity *in vivo* of BP injected with adjuvant might include activation of host macrophages to present BP lymphocytes in an immunogenic form. It has been shown that adjuvants stimulate macrophages[9]. However, it is not clear how macrophages might render BP immunogenic for initiator lymphocytes either *in vivo* or *in vitro*. Studies in this laboratory have shown that antigen extracts from syngeneic tumour cells can be immunogenic for mouse lymphocytes *in vitro* when associated with macrophages[10]. Thus, macrophages seem to have an important function in regulating the immunogenicity of "weak" self- or tumour-antigens, as well as that of extraneous foreign antigens[11].

The blocking of immunogenicity by soluble BP is compatible with the concept that non-immunogenic forms of self-antigens might help maintain natural tolerance to self by blocking lymphocyte receptors and preventing their triggering by immunogenic cell-bound forms of the self-antigens[12]. This concept was originally deduced from studies of recognition and triggering of lymphocytes by self-antigens on fibroblasts or thymic reticulum cells[12,13]. The results presented here strengthen this notion as they were obtained without use of heterologous serum which may have influenced the results of the fibroblast or reticulum cell model systems. The use of purified BP as a self-antigen argues against the possibility that sensitisation was directed against unknown viral or embryonic antigens which might have been expressed in cell culture. Furthermore, induction of autosensitisation in the complete absence of heterologous serum rules out any artefacts resulting from possible sensitisation against foreign antigens present in serum[14]. The recent experiments of Wekerle and Begemann, using an *in vitro* model of autosensitisation against testis antigens provides additional support for the concept of blocking self-antigens as regulators of self recognition[15]. It remains to be demonstrated, however, that soluble self-antigens operate physiologically *in vivo* to prevent autoimmunity by blocking recognition of the same self-antigens in immunogenic form.

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Naturally-occurring antibodies to poly(ADP-ribose) in patients with systemic lupus erythematosus

POLY(ADP-RIBOSE) is a biopolymer which is synthesised by poly(ADP-ribose) polymerase in cell nuclei[1]. Its structure and physicochemical characteristics have been reviewed[1]. Its biological function was suggested to be related to DNA synthesis in nuclei[2–4], and there are several reports on its natural occurrence[5–9]. We reported previously that a specific antibody to poly(ADP-ribose) could be produced in rabbits injected with a complex of poly(ADP-ribose) and methylated bovine serum albumin[10]. Kidwell and Mage reported on the presence of poly(ADP-ribose) in HeLa cells and the correlation of its content to cell cycles by a radioimmunoassay[11]. Recently we found that binding activities of the sera of patients with systemic lupus erythematosus (SLE) to ^{14}C-poly(ADP-ribose) were higher than those of the sera of normal individuals, and we suggest that poly(ADP-ribose) as well as antibodies to poly(ADP-ribose) occur naturally in human beings. This paper reports that binding activities of the sera of SLE patients to ^{14}C-poly(ADP-ribose) are attributable to immunoglobulins, mainly to IgG, but not to nonspecific interactions between ^{14}C-poly(ADP-ribose) and IgG; specificity of the binding was verified by inhibition tests using unlabelled poly(ADP-ribose) and 14 related compounds.

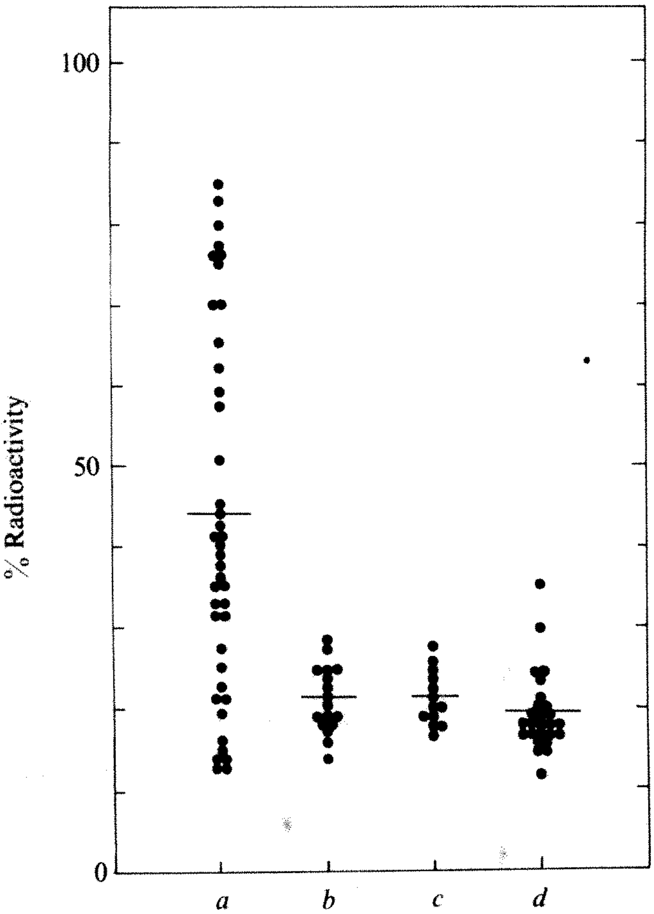
Figure 1 shows binding activities of the sera of SLE patients to ^{14}C-poly(ADP-ribose) tested by a Millipore filter method, as described in the legend to Fig. 1. The binding of the sera of SLE patients to ^{14}C-poly(ADP-ribose) was higher than that of the sera of patients with other diseases or normal individuals. It is well known that antibodies to nucleic acids occur with great frequency in SLE patients[13–15]. Poly(ADP-ribose) is a well characterised biopolymer synthesised by a nuclear enzyme[1] and highly immunogenic[10], and thus, we postulated that high binding of SLE sera to ^{14}C-poly(ADP-ribose) was due to naturally-occurring antibodies to poly(ADP-ribose).

To examine this hypothesis, crude immunoglobulin fractions obtained from five different SLE sera showing high binding activity were applied to a column of Sephadex G-150. As controls, crude immunoglobulin fractions from two sera of different normal individuals were similarly applied to a column. Figure 2 shows profiles of ^{14}C-poly-(ADP-ribose) binding in the fractions. In all SLE sera, ^{14}C-poly(ADP-ribose) binding activity was observed in the 7S as well as the 19S γ-globulin fractions. On the other hand, in the sera from normal individuals, ^{14}C-poly(ADP-ribose) binding activity was extremely low and was found only in the 19S γ-globulin fractions.

To prove that ^{14}C-poly(ADP-ribose) binding in the fraction from SLE patients was attributable to immunoglobulin itself, the double antibody method, using anti-μ and anti-γ chain sera (Behringwerge) was applied to the fraction of Fig. 2*a*, as follows. Amounts of ^{14}C-poly(ADP-ribose), and aliquots of the Sephadex fractions used were the same as in the Millipore filter method. After incubation in phosphate-buffered saline–bovine serum albumin at 37 °C for 1 h, 100 or 200 μl of anti-μ or anti-γ chain sera was added with 20 μl of normal human serum as a carrier. The mixture was further incubated at 37 °C for 1 h, and kept at 4 °C overnight. The precipitate thus formed was collected by centrifugation at 4 °C, washed once with cold PBS and solubilised in an NCS tissue solubiliser and the radioactivity was measured in a liquid scintillation counter. Profiles of ^{14}C-poly(ADP-ribose) binding obtained by the double antibody method coincided very well with those obtained by the Millipore filter method (data not shown).

In addition, F(ab′)$_2$ was isolated from the sera of SLE patients as well as a normal individual, according to the method of Nisonoff *et al.*[17,18] as described in the legend to Fig. 3. ^{14}C-poly(ADP-ribose) binding to the F(ab′)$_2$, as a function of their concentrations, is shown in Fig. 3. All the F(ab′)$_2$ from different SLE patients clearly showed ^{14}C-poly(ADP-ribose) binding, whereas there was practically no binding with F(ab′)$_2$ from the normal individual. These results indicate that the high ^{14}C-poly(ADP-ribose) binding to the sera of SLE patients is attributable to specific interactions between ^{14}C-poly(ADP-ribose) and immunoglobulin, mainly IgG.

Fig. 1 ^{14}C-poly(ADP-ribose) and unlabelled poly(ADP-ribose) were prepared by the method of Sugimura *et al.*[12]. The assay of ^{14}C-poly(ADP-ribose) binding to serum was carried out by a slight modification of the previously described method[10], as follows: A mixture of ^{14}C-poly(ADP-ribose) (3,500 c.p.m., 4 ng) and 10 µl of serum was adjusted to a volume of 1 ml with phosphate-buffered saline containing 0.1% bovine serum albumin, *p*H 7.4 (PBS-BSA), and incubated first at 37 °C for 1 h with gentle shaking followed by 1 h incubation at 0 °C. After incubation, the mixture was poured onto a 0.22-µm pore size (GS WP 025 00) Millipore filter and washed with 3 ml of phosphate-buffered saline, *p*H 7.4 (PBS) at room temperature. The Millipore filter was then dried and the radioactivity was measured, using a Toluene-based scintillator by a liquid scintillation counter (Packard). In the absence of serum, the radioactivity trapped on the Millipore filter was less than 5% of the added ^{14}C-poly(ADP-ribose). The background count was subtracted from all experimental values. The serum was incubated at 56 °C for 30 min before use. *a*, SLE; *b*, rheumatoid arthritis; *c*, patients with other diseases; *d*, normal individuals.



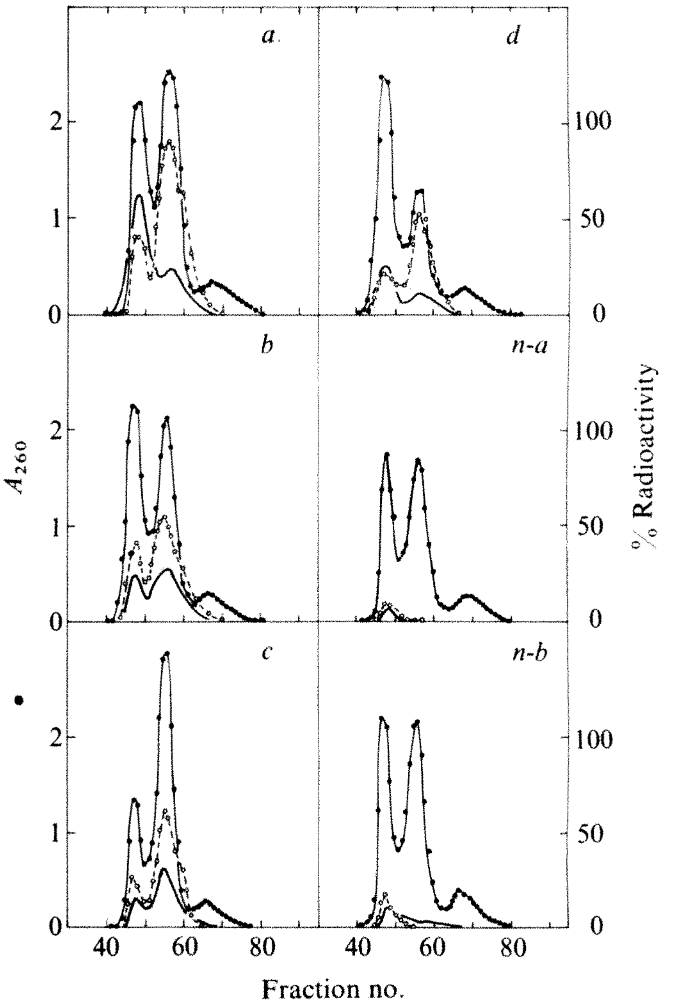


Fig. 2 Sera were diluted twice in PBS and were precipitated in half-saturated ammonium sulphate. The crude γ globulin (1.5 ml) was applied to a column of Sephadex G-150 (1.8 × 84 cm), which had been equilibrated with PBS. Two-millilitre eluate fractions were collected at a flow rate of 10 ml h^{-1}. An aliquot of each fraction (70 µl) was used for the assay of the binding to ^{14}C-poly(ADP-ribose), as described in Fig. 1. Native DNA binding in each fraction was assayed using ^{3}H-native DNA. ^{3}H-native DNA (2,700 c.p.m., 75 ng) was prepared by the method of Botchan *et al.*[16] from cultured mouse myeloma cells (L5178Y). Assay conditions were the same as for ^{14}C-poly(ADP-ribose), described in Fig. 1. Four cases out of five different SLE patients and two normal individuals are presented here. *a*, *b*, *c* and *d*, SLE patients; *n-a* and *n-b*, normal individuals. - - - O - - -, ^{14}C-poly(ADP-ribose) binding; ———, ^{3}H-native DNA binding. ●, A_{280}.

The specificity of ^{14}C-poly(ADP-ribose) binding to the 7S γ-globulin fraction, in five cases of SLE sera, was tested by inhibition tests as shown in Table 1. Inhibition of ^{14}C-poly(ADP-ribose) binding was caused partly by poly(A)·poly(U), poly(A) and poly(I), therefore the ^{14}C-poly(ADP-ribose) binding to SLE sera may represent a sum of cross reactions with antibodies to these nucleic acid forms. To clarify this, the SLE sera were subjected to absorption experiments with poly(A)·poly(U), poly(A) and poly(I). The residual binding of the ^{14}C-poly(ADP-ribose) by absorbed sera was no longer inhibited by poly(A)·poly(U), poly(A) and poly(I), but only by unlabelled poly(ADP-ribose), thereby ^{14}C-poly(ADP-ribose) binding to the SLE sera, shown in Fig. 1, seems

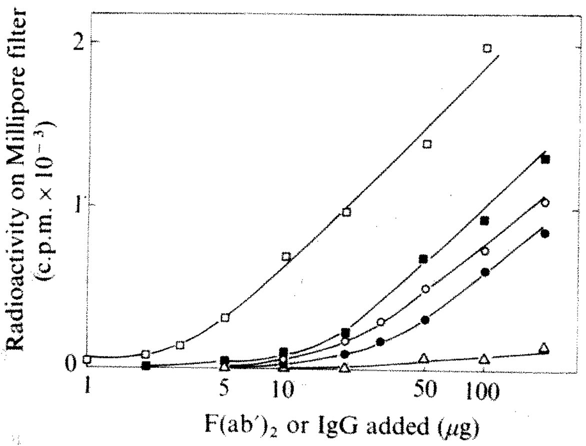


Fig. 3 IgGs of SLE patients *b*, *c*, *d* and normal individual *n-a* were purified from 7S γ-globulin fraction (2nd peak of Fig. 2) by the method described previously[10]. They were digested with 10% crystallised hog stomach pepsin (Sigma) in 0.1 M sodium acetate buffer, *p*H 4.8 at 37 °C for 18 h and neutralised with 1 N NaOH. F(ab')$_2$, thus formed, were precipitated at a concentration of 18% sodium sulphate. The precipitate was once washed with a solution of 18% sodium sulphate, and was dialysed overnight against PBS. The ^{14}C-poly(ADP-ribose) binding assay was performed by the Millipore filter method, as described in the Fig. 1 legend. □, untreated IgG from SLE patient *c*; ■, F(ab')$_2$ from SLE patient *c*; ○, F(ab')$_2$ from SLE patient *b*; ●, F(ab')$_2$ from SLE patient *d*; △, F(ab')$_2$ from normal individual.

to be immunologically specific. Variations in the degree of inhibition, shown in Table 1, may reflect the complexity as well as the state of the disease.

Furthermore, the SLE serum antibodies to poly(ADP-ribose) were most reactive to the poly(ADP-ribose) with 20 repeating ADP-ribose units which was used in the text, and the reactivity of the antibodies was dependent on the chain length of the polymer. This was also true for experimentally induced rabbit antibodies to poly(ADP-ribose).

As native DNA was not a good inhibitor (Table 1) and profiles of ^{3}H-native DNA binding in the fraction of Sephadex G-150 column were different from those of ^{14}C-poly(ADP-ribose binding (Fig. 2), antibodies to poly(ADP-ribose) seem to be completely different from antibodies to native DNA. Antibodies to poly(ADP-ribose) in some SLE patients so far examined still maintained the high levels even after reduction in levels of antibodies to native DNA. The value of the serum antibodies to poly(ADP-ribose) for the judgement of clinical state of SLE patients remains to be studied.

Table 1 Inhibition of ^{14}C-poly(ADP-ribose) binding to 7S γ-globulin fractions by unlabelled poly(ADP-ribose) and related compounds

	SLE patient				
Related compound	*a*	*b*	*c*	*d*	*e*
	% Inhibition				
Poly(ADP-ribose)	82	58	75	55	69
ADP-ribose	5	10	0	10	19
Poly(A)·poly(U)	32	20	14	12	31
Poly(I)·poly(C)	12	6	1	9	17
Poly(A)	25	0	5	2	16
Poly(U)	5	0	4	8	11
Poly(I)	14	0	13	14	19
Calf thymus DNA (native)	4	9	0	7	13
Calf thymus DNA (denatured)	9	0	0	12	11
SV40 viral DNA	0	0	0	4	4
Yeast RNA	12	3	0	7	11
E. coli tRNA	4	0	0	8	10
Rat liver ribosomal RNA	0	4	0	4	6
5'-Adenosine monophosphate	1	3	1	7	14
3'-Adenosine monophosphate	0	0	5	6	6

A 250-fold excess (1 μg) of unlabelled poly(ADP-ribose), or related compounds, was incubated with 7S γ-globulin fraction of Fig. 2 in the presence of ^{14}C-poly(ADP-ribose) (3,500 c.p.m., 4 ng), and ^{14}C-poly(ADP-ribose) binding to the fraction was measured by the Millipore filter method as described in the legend to Fig. 1. In the absence of unlabelled poly(ADP-ribose) or related compounds, samples from SLE patients *a*, *b*, *c*, *d* and *e* were adjusted to bind 65% of the radioactivity added as ^{14}C-poly(ADP-ribose).

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Potassium ion noise currents and inactivation in voltage-clamped node of Ranvier

THE experimental results of voltage noise analyses for the potassium channel system in frog nerve membranes are inconsistent with the theoretical expectations: the measured noise intensity should decrease at positive membrane potentials, where an increase is found[1,2]. The theories[3-7] are based upon the empirical Hodgkin–Huxley equations, and therefore "... cover only the short term responses of the membrane ..."[8]. Our noise experiments were made in the stationary state. A recent report suggests Hodgkin–Huxley kinetics for potassium noise currents measured earlier after changes in membrane potential[9]. Taken together these results suggest, however, that the potassium channel system changes its behaviour in time with respect to its noise pattern, which implies the occurrence of structural changes in time. We show here that the potassium channel system does indeed change structurally during long duration voltage steps.

The mean membrane current changes in time in response to a pulse of constant voltage (voltage clamp). It rises to a

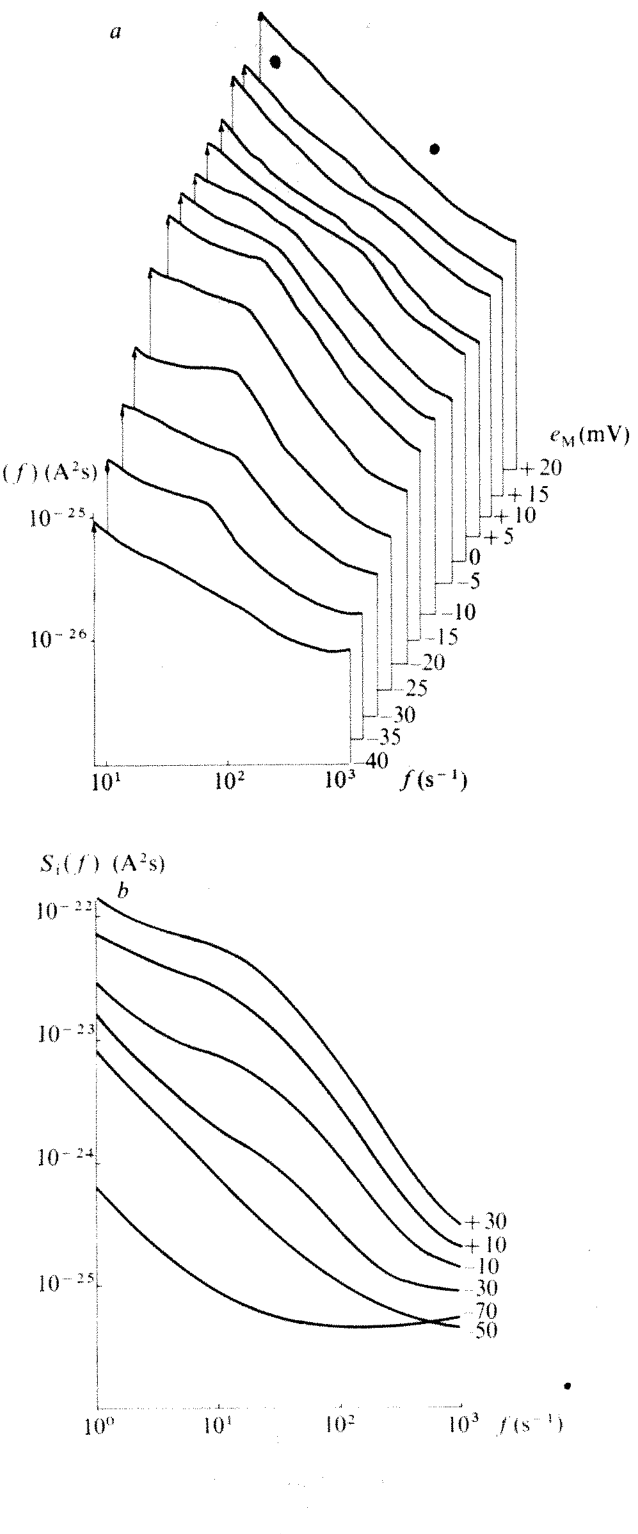

Fig. 1 Spectral densities $S(f)$ of potassium noise currents as a function of membrane potential for one node of Ranvier, at 21 °C. Phase 1 spectra in *a* and phase 2 spectra in *b*. The membrane current was filtered by a two-pole Butterworth high-pass filter with a cutoff at 5 s^{-1}. For phase 1 noise measurements 30–60 voltage steps of 600 ms each were registered per membrane potential. Each signal was further amplified (5,000 times) after a delay of 400 ms to allow the filter transients to sufficiently decay below the membrane noise level[15], and subsequently recorded on analogue tape (bandwidth 0–5 × 10^3 s^{-1}). After low pass filtering (f^{-8}, Butterworth response) the digitalised correlation function estimator, calculated by a HP3721 correlator, was multiplied with a time window after Hann and Fourier transformed. The set of 30–60 Fourier transforms was averaged for each spectrum. For phase 2 noise measurements single voltage steps of 60–100 s were used at each potential. The measurements were started 20–40 s after the beginning of a step to be in the steady state of inactivation. The spectra were obtained in a similar way, but here the averaged correlation function was used instead.

maximum within 25 ms and stays there for pulses shorter than 200 ms (refs 10, 11). Inactivation, that is, a subsequent decrease of the potassium current, occurs with longer pulses. The decrease proceeds in two phases: a relatively fast decline followed by a slower decrease to a non-zero stationary level[12].

We measured potassium noise currents both during the quasi-stationary early part of the first decline in potassium current (here called phase 1) and in the stationary state after the end of the slow decrease in current (phase 2).

Single sciatic motor fibres of *Rana esculenta* were voltage clamped in a setup after Frankenhaeuser[13], designed for low noise performance and for measurement of the absolute value of the membrane current[14]. The sodium system was blocked with TTX (tetrodotoxin, 300 nM in the bathing medium). The mean potassium current was measured with 25-ms voltage pulses, preceded by a 2-s pulse at −100 mV to remove inactivation. The currents were digitised, stored and subsequently analysed in terms of the Hodgkin–Huxley equations. Phase 1 noise measurements were made with 600-ms duration voltage pulses, with current noise analysis in the interval from 400 to 600 ms (ref 15). 60–100-s voltage steps were used for phase 2 investigations, where the noise analysis was started after complete inactivation.

Both in phase 1 and in phase 2, two noise components were found in the spectra (Fig. 1). A relaxation noise component (the humps in the spectra) and a f^{-1} noise component (with spectral intensity $S(f) = c/f$, slope −1 in the graphs). The relaxation noise component (obtained after correction for the f^{-1} noise [16]) has a constant spectral intensity $S(+o)$ at low frequencies and declines with f^{-2} at high frequencies (slope −2). The intersection between the two frequency asymptotes gives the corner frequency f_c.

The potassium channel blocker TEA (10 mM tetraethylammonium chloride in the bath) decreases both components in both phases, as expected[1,2,9]. The effect of TEA, however, differs for the two phases. The phase 1 noise intensity $S(+o)$ is about 10 times smaller than before TEA application, while the remaining intensity in phase 2 is quite large: at least 70% of its unpoisoned value. The mean potassium current behaves similarly. The initial reduction by 10 mM TEA to about 5% of the original value[17,18], is followed, however, by an increase during the period corresponding with the decrease of the potassium current (inactivation) in the unpoisoned state. The final current intensity under TEA is about 50% of its value in the untreated state. The potassium channel system of frog nodes, therefore, changes its properties quite drastically with time.

Investigation of the potassium noise properties confirms this (Fig. 2). The low frequency intensities $S(+o)$ are a function of membrane potential (Fig. 2*a*) and go through a maximum for phase 1 noise (near −20 mV). $S(+o)$ however, shows a different behaviour in phase 2, in accordance with our previous results[2]. The cutoff frequencies f_c (Fig. 2*b*) also behave quite differently with a pronounced dependence on membrane voltage[9] in phase 1 (above −10 mV) but not in phase 2[2,16] (with $f_c \simeq 45$ s^{-1} at 21 °C).

It follows that the potassium relaxation noises in phases 1 and 2 are generated by different sources. Here the frog nerve differs from the squid axon where "phase 2" noise is reported to be related to Hodgkin–Huxley potassium kinetics[19,20]. In frog nodes phase 1 noise is most probably related to the potassium Hodgkin–Huxley kinetics. We calculated the properties of the potassium relaxation noises according to the simplest translation of the Hodgkin–Huxley equations in stochastic terms[3,4], using the Hodgkin–Huxley parameters from the 25-ms voltage clamp experiments. The results (lines in Fig. 2) show that the measured phase 1 intensities $S(+o)$ follow the predicted relation quite well. This parameter may be less sensitive for small deviations from Hodgkin–Huxley kinetics than the kinetic parameter f_c, which shows a deviation. Here partial inactivation present in phase 1, although small, may also have a role. The potassium channel conductance γ_K was cal-

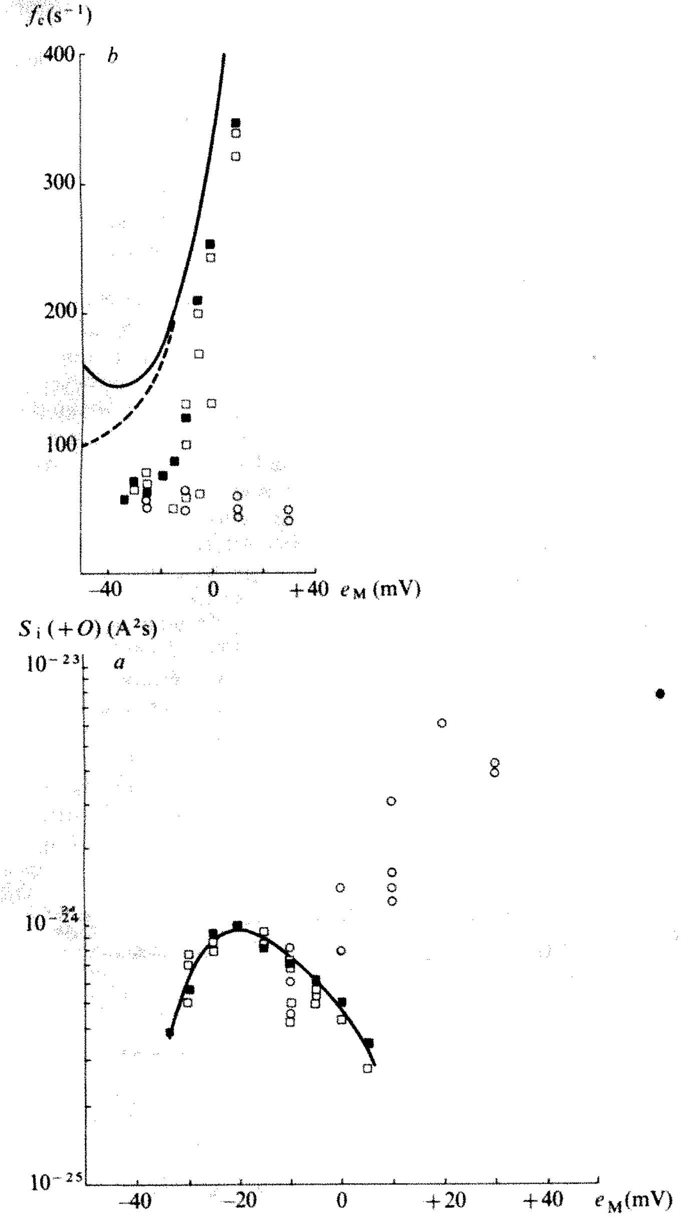

Fig. 2 *a*, Low frequency intensities $S(+o)$; *b*, cutoff frequencies f_c, of the potassium-relaxation spectra of four nodes of Ranvier, at 21 °C, plotted against membrane potential, for noise currents recorded in phase 1 (□) and in phase 2 (○). Lines give theoretically expected values for Hodgkin–Huxley four-gate open–close kinetics (solid), with the voltage clamp parameters of one of the nodes (■); dashed line is $f_c = \dfrac{1}{2\pi\tau_n}$

culated, assuming that all channels are in the open state when the mean potassium conductance $\langle g_K\rangle$ reaches its maximal value $\bar{g}_K$ at a certain positive membrane potential. The channel conductance then follows from[3,7]:

$$\gamma_K = \frac{\sigma_I^2}{\langle I_K\rangle(E-E_K)(1-\langle g_K\rangle/\bar{g}_K)} \qquad (1)$$

with mean potassium current $\langle I_K\rangle$, membrane potential E and potassium equilibrium potential E_K. The variance of the current fluctuations, σ_I^2, was obtained by integration of the one-sided spectrum. This gave the estimate for $\gamma_K = 2.9 \pm 0.4$ pS (three nodes, 12 estimates, at 21 °C). This value, which is close to that reported by Begenisich and Stevens[9] (4.0±0.27 pS, 14 observations at 15 °C) does not depend on membrane potential within its measurable range (−30 to +5 mV).

The subsequent change in behaviour must be due to either conformational changes of the channel system or a new potassium channel system arising during inactivation. Which possibility actually occurs must be left open. With the conformational change assumption, however, the channel conductance in phase 2 can be calculated with equation (1). The phase 2 channel conductance now seems to depend strongly on membrane potential, increasing more than twice in the range from −10 to +30 mV. This implies that equation (1) does not yield the open channel conductance and necessitates the use of a specific channel model[7].

Our results prove that the potassium channel system in frog nodal membrane changes its properties profoundly during long duration voltage steps. Another new result, the difference in pharmacological behaviour with regard to the passage of time after the application of a voltage step, shown here to exist for TEA, may also have practical implications. We thank Dr J. de Goede and Professor Dr A. A. Verveen for advice and support and Dr H. D. van Dorp for the computer program. We also thank W. C. Deegenaars, J. H. Kussendrager and A. de Vos for technical assistance. This work was supported by a grant from The Netherlands Organisation for the Advancement of Pure Research (ZWO).

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Bioluminescence of lantern fish (Myctophidae) in response to changes in light intensity

THE characteristic ventral distribution of fish photophores suggests that their luminescence reduces the silhouette when viewed against a background of downwelling light[1–3]. This hypothesis is supported by demonstration of direct neural control of photophores in several species of myctophids and by morphological and behavioural indications that *Tarletonbenia crenularis* matches its luminescence to background illumination by visually monitoring a supraorbital photophore[4,5]. There is similar evidence for other taxa: shallow water leiognathids emit light in response to brief photic stimulation and mid-water squid have photosensitive vesicles which may participate in countershading[6–8]. Here we report photometric measurements of myctophid bioluminescent responses to light levels comparable with those occurring in their environment.

Symbolophorus californiensis (Eigenmann and Eigenmann) bears approximately 70 photophores of about 0.5 mm diameter on ventral and lateral surfaces. When immature, it lacks the brilliantly flashing luminous patches characteristic of many myctophids. It migrates daily from 600 m to or near the surface[9]. Immature specimens in good condition were netted at night by neuston net from RV Velero IV over the San Clemente Basin. They were transferred to containers of surface temperature seawater and held in darkness for experimentation within the next several hours aboard ship. Individuals were placed in a 1-l cylindrical glass jar in an apparatus with which it was possible to continuously record bioluminescence against controlled background irradiance of up to 0.09 μW cm^{-2}. Background irradiance was generated by five Sylvania R1166 glow modulator tubes emitting white light onto a diffusing hemisphere above the specimen chamber. An EMI 9781B photomultiplier, viewing the entire specimen chamber from below, detected bioluminescence. Interference from background irradiance was eliminated by pulsing the glow modulators at 180 Hz in synchrony with a rotating shutter over the photomultiplier tube. The 'on' period for the glow modulators corresponded to the period during which the photomultiplier tube was covered by the shutter. The photomultiplier signal was processed by a Kiethley 840 lock-in amplifier and displayed on a chart recorder with a bandwidth of 70 Hz. Background irradiance was measured with a United Detector Technology 40X radiometer. Tests of the performance of the apparatus showed that a photophore-sized light source, placed 10 cm from the photomultiplier, and producing an estimated irradiance of 5×10^{-5} μW cm^{-2}, could readily be detected against a 0.5-m^2 background producing an irradiance of 8×10^{-2} μW cm^{-2}. Because the 180-Hz glow modulator cycle exceeds the highest visual flicker fusion frequency reported for fish, the apparatus makes it possible for a subject to be exposed to perceptually continuous light while being monitored by a photomultiplier protected from dark current and sensitivity changes caused by excessive light exposure of the photocathode[10].

Six *S. Californiensis*, 26–30 mm standard length, responded similarly to repeated exposures to various background light regimes. Latency of bioluminescence initiation after switching on background lighting was as little as 0.5 s, with averages for four fish being 18.5 (four tests), 7.0 (one), 1.0 (four) and 2.0 s (six). 'Off' responses were similarly rapid but the data cannot be used for a precise measurement. In many instances a probable luminescent startle response of 0.5–1.0 s latency, requiring an average of 4 s from half amplitude on to half amplitude off, precede initiation of sustained bioluminescence (Fig. 1*a* and *b*). Our data cannot be used to estimate how closely the fish match the background irradiance with their photophore emission because the position of the fish is indeterminate. The level of bioluminescence, however, is clearly directly related to background irradiance over part of the background irradiance range. When exposed to a ramp of increasing irradiance, ranging from near 0 to 0.008 μW cm^{-2} over 55 s, bioluminescence also increased in ramp-like fashion (Fig. 1*a*). Assuming a nominal position of 10 cm from the photomultiplier and maximum exposure of the ventral surface of the fish to the photomultiplier, point A in Fig. 1*a* corresponds to an irradiance of 2×10^{-5} μW cm^{-2} at the photocathode of the photomultiplier tube. Further evidence of proportionality of bioluminescence to background light intensity is seen in Fig. 1*b* and *c* where bioluminescence is off scale at 0.05 μW cm^{-2} background and considerably less at 0.002 μW cm^{-2} background. Bioluminescence is inhibited when the background reaches about 0.09 μW cm^{-2} and reappears when the background is reduced to 0.02 μW cm^{-2}, an effect that may be due to visual light adaptation because bioluminescence can be maintained to 0.08 μW cm^{-2} background irradiance when the background is cycled from dim to bright.

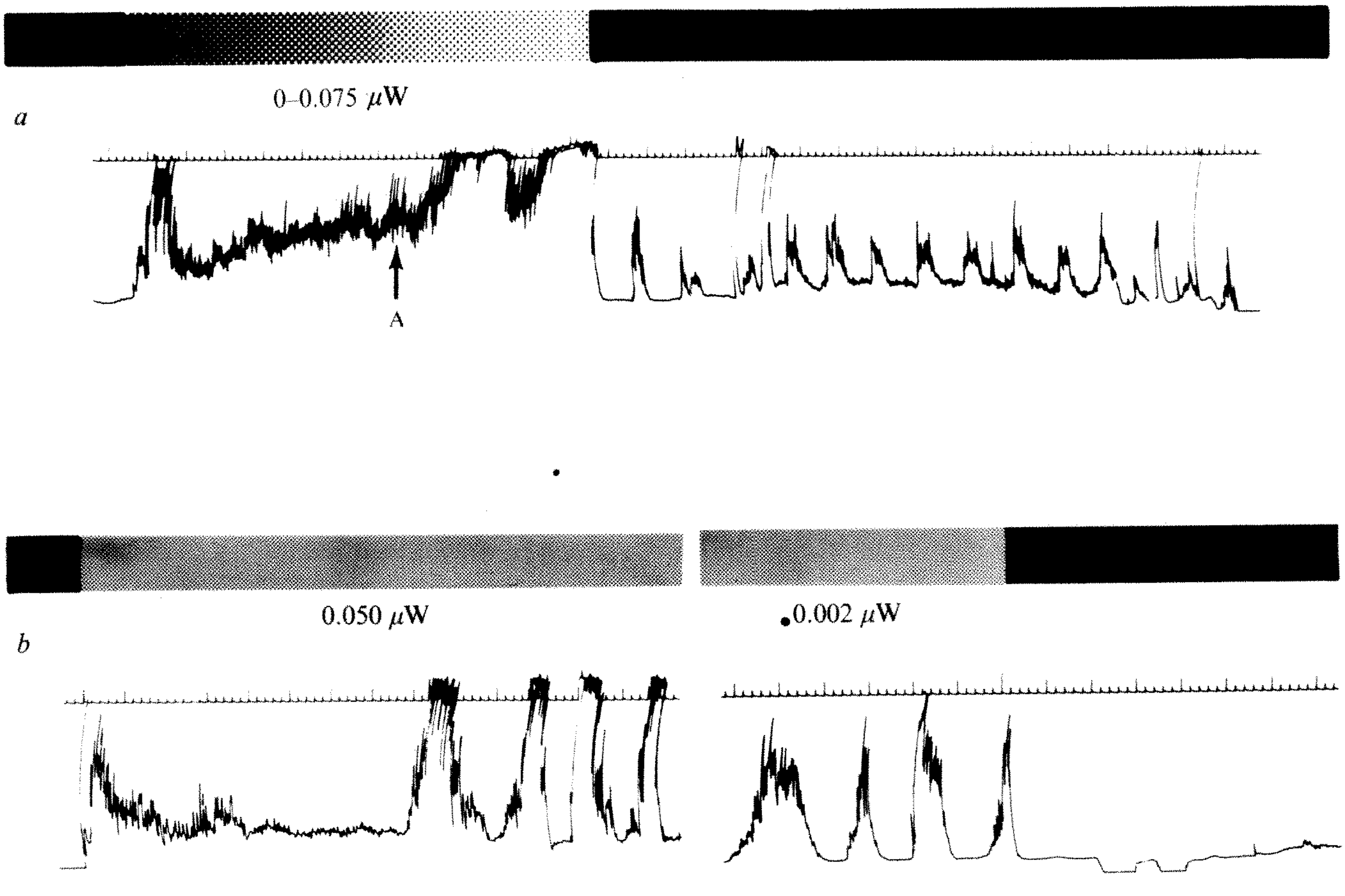

Fig. 1 Photomultiplier recordings of *Symbolophorus* bioluminescent responses to variation in background illumination. *a*, Probable alarm response and gradually increased bioluminescence in response to gradual increase in background illuminance, followed by diminished bioluminescence in darkness (point A equals 2×10^{-5} μW cm^{-2} at photomultiplier); *b*, probable alarm response followed by delayed onset of presumed countershading bioluminescence on sudden increase in background illuminance; *c*, sudden cessation of luminescence on cessation of low-level illumination. Records (*b*) and (*c*) were made with the same specimen. Time marks at 1 and 5 s, increasing bioluminescence indicated by upward deflection of traces.

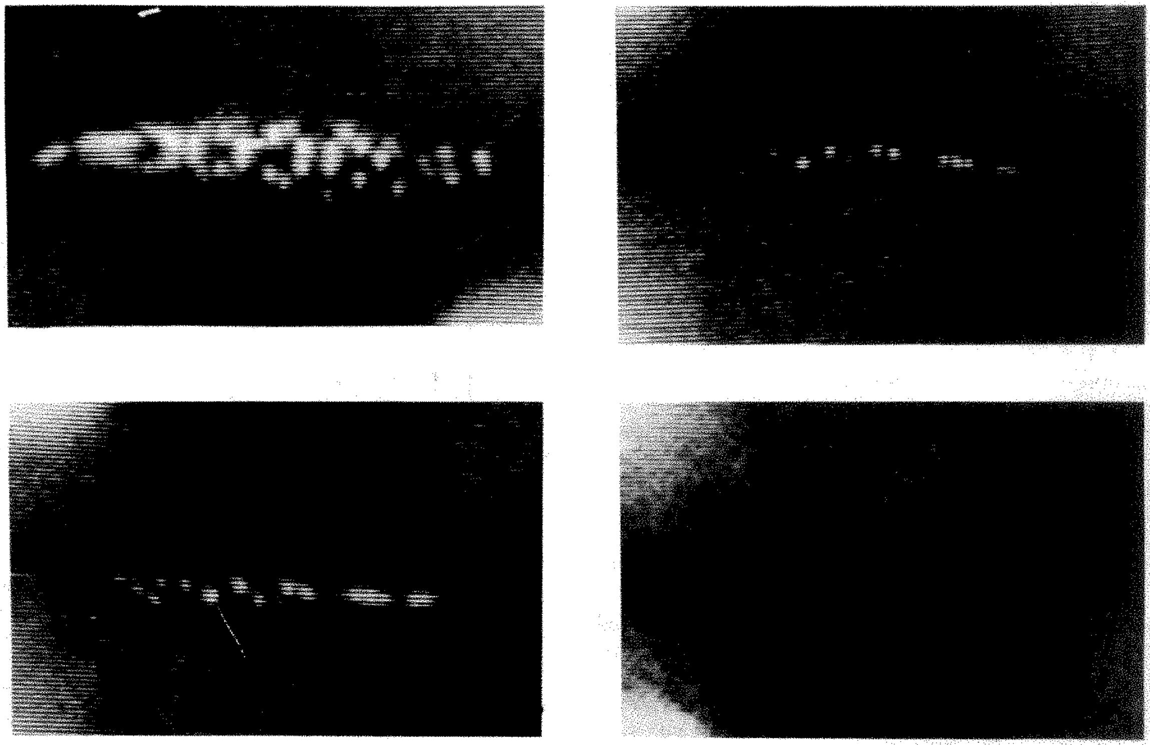

Fig. 2 Image intensified video recordings showing rapid onset of luminescence from ventral photophores of an approximately 5-cm standard length, unidentified myctophid. Interval between first and third frames is 116 ms, 676 ms for the entire series.

During continuous background exposure, bioluminescence continues for at least 15 min, our longest test at a single background irradiance level. The short term peaks (roughly six to eight per min) coincide with the ship's motion. We believe they represent rotation of the free-swimming specimen with reference to the photomultiplier and that bioluminescence during exposure to light is uniform. A similar effect can be produced with an artificial light source in place of the fish. Photomultiplier records (Fig. 1*a*) and visual observations show some low level bioluminescence in darkness, often due only to the slow winking on and off of a single photophore.

Behaviournally-induced photophore responses of myctophids may be more rapid than the 0.5 s measured with the relatively poor time resolution of our recording system, as suggested in image intensified video recordings made according to the method of Barnes and Case[4]. Figure 2 shows video frames of an unidentified myctophid obtained by trawling in the Banda Sea. Within 677 ms all photophores in view have markedly increased their output.

These experiments demonstrate the presence of the requisite visual sensitivity linked with photophore control to facilitate bioluminescent reduction of the silhouette (countershading or counterillumination) in the typical light regime of lantern fish. Both the rapidity of photophore control and the demonstration of what may be a transient alarm response, however, argue that myctophid photophores have other roles.

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Voltage signal of photoreceptors at visual threshold

IT has long been known that vertebrate photoreceptors can signal the absorption of single quanta of light[1]. The extraordinary sensitivity of the visual system is usually ascribed both to the sensitivity of the photoreceptors and to the integration of their signals by higher-order neurones. A more complete explanation of visual sensitivity could be given if it were possible to record the response of receptors at the visual threshold. Unfortunately, it has not been possible to do this at the absolute threshold, largely because of interactions between receptors[2]. Near the absolute threshold only a small proportion of the receptors absorb quanta, but the photocurrent from these cells spreads through electronic synapses[3] to many other cells. Thus the voltage responses in the receptors are non-uniform, and it is not possible to tell which responses contribute to the threshold. Fortunately this difficulty does not arise at the increment threshold since in the presence of bright background light, the variation in response from one receptor to the next is minimal. The differences among the receptors in the number of quanta caught will be much less important than at absolute threshold, and the connections between the receptors will tend to equalise their voltages. In this report we compare receptor responses to behavioural responses in the light-adapted turtle and show that the receptor response at threshold is only a few microvolts in amplitude.

The amplitude of turtle receptor responses to flashes of light in the presence of bright backgrounds can be estimated from the results of Baylor and Hodgkin[4]. The sensitivity of red-sensitive cones in the turtle *Pseudemys scripta elegans* varies with the intensity of background light according to the relation

$$\frac{1}{S_F} = \frac{1}{S_F^D} + \zeta I_B \qquad (1)$$

where S_F is the sensitivity of the cones to brief, increment flashes, S_F^D is the cone sensitivity in the dark (that is, with no background light), I_B is the intensity of the background, and ζ is a parameter having the nearly constant value of 20 s V^{-1} per flash. Near threshold the peak amplitude of the receptor response is equal to the product of the increment flash intensity and S_F, the flash sensitivity[4-6]. Thus the voltage signal (ΔV_T) at the increment threshold (ΔI_T) is given by

$$\Delta V_T = S_F \Delta I_T \tag{2}$$

As long as ΔV_T is invariant with background intensity, equations (1) and (2) can be combined to give the Weber-Fechner relation for the behavioral threshold

$$\Delta I_T = k(I_B + I_0) \tag{3}$$

with I_0 equal to $\Delta V_T / k S_F^D$ and the Weber fraction, k, equal to $\Delta V_T \zeta$. Since turtle vision has been shown to obey the Weber–Fechner relation[7,8], a determination of k can be combined with the previously determined value for ζ to give an estimate of ΔV_T.

To estimate the amplitude of ΔV_T, we have measured increment thresholds for *Pseudemys scripta elegans*, the same species of turtle used by Baylor and Hodgkin[4,5]. The apparatus has been described previously[9], but there are some alterations to be noted. The test target consisted of a circular piece of milk glass 4.9 mm in diameter, illuminated by tungsten light from a d.c. source. The test target subtended a visual field of 2°21′ and produced an image diameter size of nearly 300 μm on the retina, as estimated from a schematic eye developed for this animal by D. P. M. Northmore (personal communication). The unattenuated intensity of the white test light was measured with a calibrated thermopile to be 13.0 W m^{-2}. Background light was afforded by a second lamp similar to the test lamp mounted outside the rear of the chamber, whose unattenuated intensity was 177.5 W m^{-2}.

The procedure required a head withdrawal response in order to avoid electric shock. The experimental sequence was under programmed control, and details of training and testing have been reported previously[9]. Stimuli were presented at one minute intervals against a dark background and backgrounds of varying intensity levels. Thresholds were determined by a tracking method such that a response on any trial caused the next stimulus to be reduced in intensity by 0.1 logarithmic unit, and the absence of response caused a like increase in intensity. Determined thresholds were very reliable: The calculated mean 95% confidence interval over the series of thresholds collected was only ± 0.08 logarithmic unit.

The results of the increment threshold measurements are shown in Fig. 1. The data have been fitted with equation (3), using a value for k of 10^{-4} s per flash. Since in light-adapted *Pseudemys* the spectral sensitivity of the behavioural response which we have used for our measurements is in close agreement with the spectral sensitivity of the red-sensitive cones[5,9] we will assume that the curve in Fig. 1 reflects primarily the activity of the long-wavelength cone system. It is doubtful that green- or blue-sensitive cones contributed to our measurements, since the white light of the test and adapting beams had most of its energy in the red region of the spectrum. In any case, small contributions from green-sensitive cones would not affect the calculation of ΔV_T, since ζ is approximately the same for both green- and red-sensitive receptors[4].

From the values of k and ζ, we calculate that ΔV_T is approximately 5 μV. Although there is some variability in our measurements, we estimate that this value is accurate to within 0.3 log unit, so that ΔV_T is at most 10 μV. There are several reasons to believe that this value is an upper limit for the threshold signal. First, the target used in the behavioural measurements was larger than that used by Baylor and Hodgkin[4] and probably produced significant responses in horizontal cells[10]. Horizontal cells in turtle inhibit cones[11], and it is possible that this inhibition was large enough in the behavioural measurements to increase k, thus overestimating ΔV_T. Second, we do not know that our test target was optimum for eliciting responses from turtles. Finally, the intracellular measurements of cone responses were most probably made in the nuclear region of the cell body. The voltage in the synaptic pedicle is probably somewhat smaller.

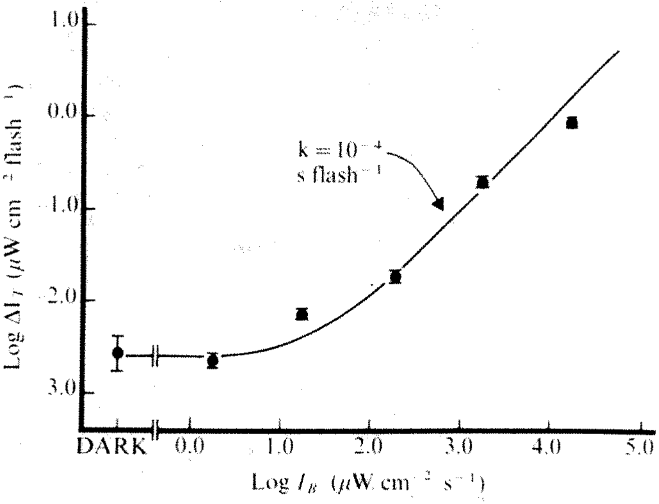

Fig. 1 Increment-threshold curve for the behavioural response of the turtle, *Pseudemys scripta elegans*. Thresholds measured with flashes between 10 and 50 ms have been corrected for the difference in flash lengths and averaged together, since the behavioural threshold in turtle shows complete temporal summation for flashes up to at least that level (Granda and Maxwell, unpublished data). Data points give the mean threshold and bars, one standard deviation. Points have been fitted with equation (3) using a value for k of 10^{-4} s per flash.

Our results show that a receptor response of no more than 10 μV is sufficient to signal visual detection. This means that a very small voltage can change the flow of synaptic transmitter between receptors and second-order neurones, suggesting that the sensitivity of transmission at the receptor synapse makes an important contribution to the sensitivity of photodetection. It is of some interest that the threshold signal in photoreceptors appears to be comparable in magnitude to the signals estimated to produce threshold responses in electroreceptors[12]. Both photoreceptors and electroreceptors make synapses with second-order neurones which are characterized by presynaptic specializations including a synaptic ribbon or bar and an arciform or presynaptic density[13] which may function to direct the release of synaptic transmitter[14,15]. The similarity in the microanatomy of the synapses in these two systems suggests that the characteristic presynaptic specialisation at receptor synapses may serve to permit the signalling of small amplitude responses.

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Unusual resistance to ultraviolet light in dark phase of blue-green bacterium *Anacystis nidulans*

THE role of visible light in the repair of ultraviolet light-induced damage has been extensively studied in various organisms[1]. In blue-green bacteria, photoreactivation has been found to be extremely efficient[2]. Indeed, in these organisms, which generally need light as a growth factor, the presence of an efficient photoreactivation mechanism hinders the detection of alternative repair steps which may be present. In spite of this difficulty, however, the existence of light-independent (that is, dark) repair has been observed in blue-green bacteria as suggested by the isolation of mutants with altered ultraviolet light sensitivity[3,4]. Here we report an interesting observation suggesting the presence of a very efficient repair system against damage induced by ultraviolet light (254 nm) in an obligate photoautotrophic blue-green bacterium *Anacystis nidulans*. We show that this repair system is either inhibited or less effective under aerobic conditions in the presence of light.

Synthetic medium, C-Mn, both for liquid and agar plate, was used for growth of *Anacystis nidulans* as described by Van Baalen[5]. Liquid cultures were grown at 36 ± 0.5 °C in a water bath shaker and at a light intensity of 200 foot candles from tungsten lamps. In the exponential phase, the doubling time under these conditions of growth was 5 h. Agar plates with cells spread by glass rod were incubated in tungsten light at an intensity of 200 ± 10 ft cd and at 35 ± 1 °C for 5 to 6 d after which colony counts were taken. Under these conditions of growth 100% plating efficiency was obtained.

Cells preincubated in the dark acquired a high resistance to ultraviolet light as compared to cells kept in the light (Fig. 1). This was observable in conditions when the repair by photoreactivation was reduced to minimum. It has been shown that after about 18 h of dark incubation following ultraviolet light exposure no significant change in survival can be effected by photoreactivating light, indicating the presence of only insignificant photoreactivable damage[4]. In order to minimise the repair by photoreactivation, we therefore maintained 24 h of post-exposure dark incubation at room temperature (25 °C). Since photorespiration is blocked in dark incubated cells we thought it would be interesting to see if blocking the total respiration could change the sensitivity[6]. Indeed, when the cells were incubated in light in anaerobic conditions (that is, in an argon atmosphere), the ultraviolet resistance was restored considerably in them (Fig. 1, curve *L*₁).

Significant metabolic differences have been found to occur in light and dark phases of *Anacystis nidulans*. In their studies on macromolecular synthesis, Hayashi *et al.* showed that all species of RNA synthesis continued for at least 4 h in the dark in contrast to DNA synthesis which is stopped in the dark[7]. It is also interesting to note that in the dark this organism accumulates relatively larger amounts of two stable RNA species whose biological functions are still unknown[8]. These are also synthesised in the illuminated cells but are unstable in them. We have observed that when dark incubated cells are transferred to light the ultraviolet resistance is lost in about 1 h, a period within which these stable RNA species have been reported to disappear in light[8]. We have also observed that dark incubated cells when exposed to visible light immediately after ultraviolet light treatment are more sensitive than those not receiving the visible light exposure. This dose of visible light, however, causes the recovery of a very significant fraction of cells exposed to ultraviolet light which are preincubated in light in aerobic conditions and are extremely sensitive to ultraviolet light (our unpublished data). In view of these

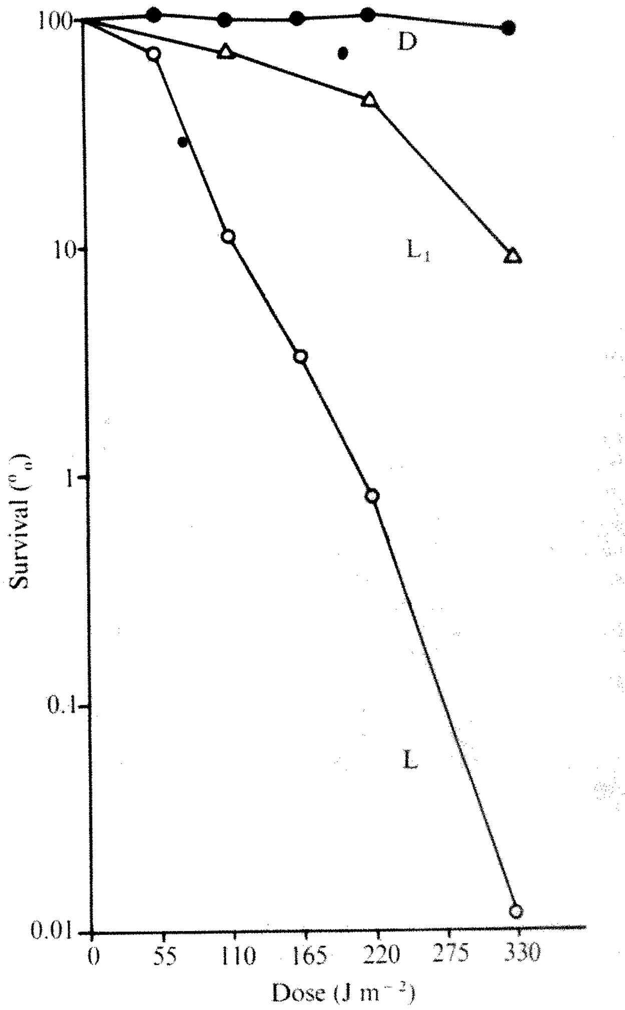

Fig. 1 Survival by *Anacystis nidulans* of exposure to ultraviolet light. Exponential phase cultures of *Anacystis nidulans* were washed with nitrate-free C-Mn medium and resuspended in the same medium at 10^7 – 10^8 cells ml^{-1}. The suspension was divided into three fractions. One fraction was kept in the dark by covering the flask with aluminium foil (D). The second fraction was kept in a vial with rubber cap, sealed with wax and was sparged with argon gas for 30 min (L_1). The third fraction was kept in an uncovered flask (L). All the samples were transferred within 40 min of washing to a water bath shaker at 36 ± 0.5 °C. L and L_1 received tungsten light at an intensity of 200 foot candles. After 24 h of incubation the cells from L, L_1 and D samples were diluted appropriately in C-Mn nitrate-free medium and spread on synthetic agar plates with a sterile glass rod under dim light. Plates with cells were exposed to ultraviolet light (254 nm) from Philips TUV 15 W germicidal lamp at an intensity of 11 J m^{-2} s^{-1} as measured by an Eppley 12 junction linear thermopile. The control and the treated plates were kept in the dark for 24 h at room temperature (25 °C). After the dark incubation all the plates were transferred to light (200 ± 10 ft cd) at 35 ± 1 °C. At the end of incubation in light for 5–6 d the colony counts were taken.

observations we suggest the existence of certain repair steps which are either more efficient in the dark or inhibited in the light under aerobic condition prior to ultraviolet light exposure. The possibility that this differential ultraviolet light sensitivity is due to differences in the nature and extent of primary damage in the light- and dark-incubated cells cannot be totally ruled out by these observations. That this effect is due to the DNA replication inhibition in the dark incubated cells seems improbable. This is because in our experiments both light- and dark-incubated cells were suspended in a nitrate-free medium for 24 h before ultraviolet light exposure. *Anacystis nidulans*, not being a nitrogen-fixing organism, does not grow in the absence of nitrate.

The presence of any repair system against damage induced by ultraviolet light which may either be induced in the dark or inhibited in light is not known. The reverse has, however, been reported. It has been shown that a large, free-living protozoan *Amoeba proteus*, grown in the dark, acquires sensitivity in its G_2 phase to near ultraviolet (365 nm) light as compared with cells cultured in 12 h light/12 h dark cycle[9]. Resistance is restored in dark-grown cells within 10 d of transfer to light/dark cycle. It has also been shown that the *Nicotiana tabacum* plant kept in dark for 7 d lose their capacity to photoreactivate tobaco mosaic virus RNA treated with ultraviolet light[10]. This capacity can be restored by transferring the plant to light.

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Evidence for nuclear antigens in cytomegalovirus-transformed human cells

THE anti-complement immunofluorescence (ACIF) method has revealed an antigen localised in the nucleus of both producer and nonproducer cultures of lymphoblastoid cells carrying the Epstein–Barr virus (EBV) genome[1]. Early nuclear antigens resembling the Epstein–Barr nuclear antigen (EBNA) have been detected in human embryo lung (HEL) cells as early as 3 h after infection with human cytomegalovirus (CMV)[2]. These early antigens could be detected only by ACIF staining. We have reported[3] that hamster embryo fibroblasts (HEF) transformed with ultraviolet-inactivated CMV exhibit cytoplasmic and perinuclear CMV-specific antigens when tested with CMV-immune sera in the indirect immunofluorescence (IF) test. We later noted that a human CMV of genital origin established a long-term persistent infection in HEL cells *in vitro*, which resulted in the development of two transformed cell lines after several cell culture passages[4]. CMV-specific antigens were demonstrated on the surface of the cells by the indirect IF technique. We have now investigated whether EBNA-like nuclear antigens can be detected in the CMV-transformed human cells with the ACIF technique, and whether there is a correlation of reactivity with early antigens of CMV-infected cells.

Monolayers of HEL cells were grown on coverslips in Dulbecco's medium supplemented with 10% foetal calf serum. The CVM-transformed human cell lines CMV-Mj-HEL-1 and CMV-Mj-HEL-2 and the tumour cell line CMV-Mj-HEL-2, T-1 were established as before (ref. 4 and our unpublished work with J. Kreider). These cells were grown on coverslips in Ham's medium supplemented with 20% foetal calf serum, 0.075% sodium bicarbonate, penicillin (100 IU ml^{-1}) and streptomycin (100 μm ml^{-1}). The C-87 strain of CMV was used for the inoculation of HEL cells at a multiplicity of 0.3 plaque-forming units (PFU) per cell. Cell sheets were fixed 3, 6 and 48 h after inoculation as described before[2]. As a control, the HIL strain of herpes simplex virus type 1 (HSV-1) and the 333 strain of herpes simplex virus type 2 (HSV-2) were used at a multiplicity of infection of 6.0 PFU per cell. Cell sheets were fixed with acetone-methanol 6 h after infection.

Human sera were obtained from hospital patients and staff members of our department. Sera of patients with CMV mononucleosis were supplied by Dr W. Henle of Children's Hospital, Philadelphia. HSV-2-specific hamster serum had an IF antibody titre to HSV-2-infected cells of 1 : 32.

HEL cells, CMV-infected HEL cells or CMV-Mj-HEL-1 cells (10^8 per ml) were used for the adsorption of the sera. Cells were added to undiluted sera, sonicated and incubated for 1 h at 37 °C and then overnight at 4 °C. The cell debris was removed by high-speed centrifugation.

Anti-human and anti-hamster immunoglobulin G fluorescent conjugate (goat) and fluorescent conjugates for anti-human complement staining (goat) were purchased from Cappel Laboratories, Downingtown, Pennsylvania. Fluorescein isothiocyanate conjugates were adsorbed with 10^8 target cells per ml before use. CMV-negative and HSV-negative human sera were used as the source of complement in the ACIF tests. Standard procedures[2] were used for IF and ACIF tests.

The following results were obtained. Eight of thirteen CMV-immune human sera reacted with nuclear antigens of CMV-Mj-HEL-1 cells. Depending on the immune serum used, 6–80% of the cells demonstrated nuclear antigens (Table 1). The reaction observed was intense and located entirely within the nuclei (Fig. 1*a*). The intensity varied from test to test and among cells within a test, and thus may have depended on the stage of the cell cycle. Occasionally, faint nuclear fluorescence was detected in transformed cells stained by the indirect IF method (Fig. 1*b*), where a more granular pattern was observed. The immune serum did not react with control HEL cells. The CMV-negative human serum, which was the source of the complement in these tests, did not react with the transformed cells (Fig. 1*c*).

There was a correlation between the CMV antibody titre of the immune sera and their reactivity with the nuclear antigens; CMV-negative human sera did not react with the nuclei of the CMV-transformed cells (Table 1). All immune sera with antibody titre higher than 1 : 64 against CMV-infected cells reacted with nuclear antigens of the transformed cells (Table 1). None of the nuclear-antigen-reactive sera reacted with nuclear antigens of control HEL or bladder cancer cells (Table 1). The antibodies to the nuclear antigens of CMV-transformed cells sometimes seemed to develop in the convalescent phase of the infection in some

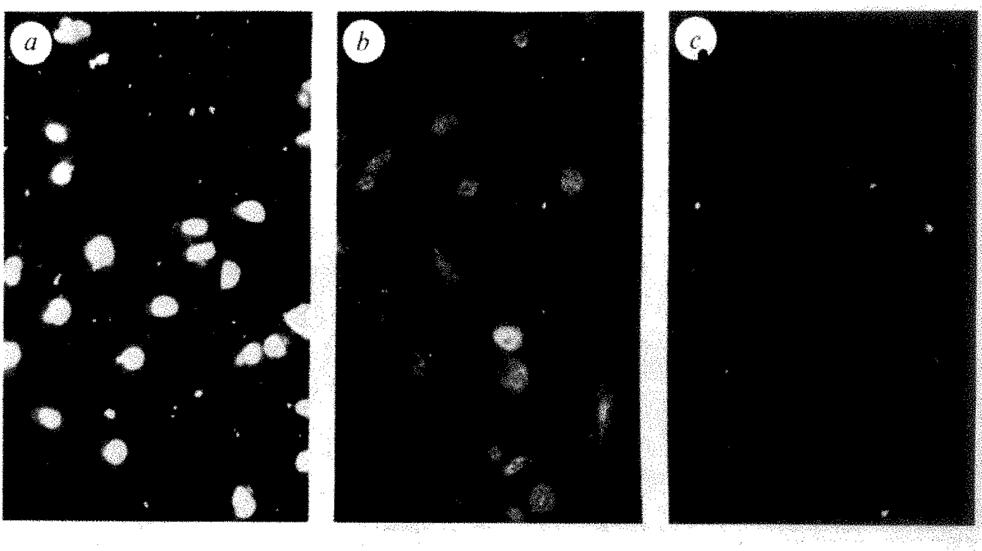

Fig. 1 *a*, Nuclear antigens in CMV-Mj-HEL-1 cells. CMV-immune human convalescent serum was used with human complement and fluorescein isothiocyanate-conjugated anti-human complement goat serum in an ACIF test. *b*, Nuclear antigens in CMV-Mj-HEL-1 cells after reaction with CMV-specific human convalescent serum and fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G. Note faint staining of antigens with the indirect IF method. *c*, CMV-Mj-HEL-1 cells were reacted with negative human serum used as a source of complement and fluorescein isothiocyanate-conjugated anti-human complement goat serum in an ACIF test (×500).

cases of CMV mononucleosis (Table 1, see sera 2 and 3). All sera that reacted with the nuclear antigens of the transformed cells also reacted with the early (6-h) antigens of CMV-infected cells. Only eight of 13 'early-antigen-reactive' immune sera, however, reacted with nuclear formed cells in the ACIF test.

Adsorption of serum with uninfected HEL cells did not influence the reactivity of the serum with the nuclear antigens of CMV-transformed cells or CMV-infected HEL cells. When adsorbed with CMV-infected HEL cells, however, the immune serum lost its reactivity against the nuclei of transformed and virus-infected cells. The reactivity with HSV-infected cells was not influenced. The adsorption of the CMV-immune serum with the CMV-transformed human cells removed the reactivity with the "early" antigens of the CMV-infected HEL cells and decreased the reactivity with the CMV-infected cells fixed 48 h after infection. HSV-2-immune hamster serum did not react with the nuclear antigens. Sera that were positive for EBNA did not react with CMV-infected or CMV-transformed cells in the ACIF test.

Some of the immune sera reacted with both nuclear and perinuclear antigens, or with only nuclear or perinuclear antigens in the transformed cells. The perinuclear reaction requires further study.

Thus intranuclear CMV-related antigens have been demonstrated in CMV-transformed human cells by means of the sensitive ACIF method. The staining of the nuclei of the transformed cells by ACIF correlated with the presence in the sera of CMV antibodies against late-phase intracellular and 'early' intranuclear antigens of CMV-infected cells. Not all immune sera with anti-CMV antibodies, however, reacted with the transformed cells. This is not surprising in view of the numerous CMV antigens that may elicit the immune response in man. The nuclear reactivity seems to be possessed primarily by immune sera, taken from patients with prostatic cancer or those in the convalescent phase of an acute CMV infection, with a CMV antibody titre higher than 1 : 64 as measured by the direct IF test. There is apparently a relationship between the nuclear antigens of the transformed cells and the 'early' 6-h nuclear antigens of CMV-infected cells. Thus, the nuclear antigens of the transformed cells may be CMV-specific T antigens of CMV-transformed human cells and may be important in immunological studies of the role of CMV in human malignancies. This study was supported

Table 1 Reactions of CMV-positive and CMV-negative human sera to ACIF* tests with antigens of CMV-infected and CMV-transformed cells

Serum no.	Diagnosis	IF antibody titre against CMV-infected HEL cells (48 h)	ACIF nuclear reaction with HEL cells	ACIF nuclear reaction with CMV-infected cells (6 h)	ACIF nuclear reaction with CMV-Mj-HEL-1 cells
1	CMV-M†	1/10	–	+	80‡
2	CMV-M§	1/32	–	+	–
3	CMV-M¶	1/32	–	+	23
4	CMV-M§	1/4	–	+	–
5	CMV-M¶	1/64	–	+	–
6	CMV-M§	1/256	–	+	6
7	CMV-M¶	1/256	–	+	9
8	CMV-M§	1/128	–	+	21
9	CMV-M¶	1/128	–	+	21
10	CMV-M§	1/32	–	+	–
11	CMV-M¶	1/32	–	+	–
12	PCa**	1/256	–	+	10–20
13	PCa	1/256	–	+	25–30
14	Normal	< 1/4	–	–	–
15	Normal	< 1/4	–	–	–

*Anti-complement immunofluorescence.
†CMV mononucleosis.
‡Percentage of fluorescing cells.
§Acute phase serum.
¶Matched convalescent phase serum.
**Prostatic cancer.

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Radiation-induced nondisjunction in oocytes of aged mice

SOME human epidemiological studies[1–4] suggest that there may be an association between nondisjunction and preconception exposure to abdominal X rays in women. This response to radiation appears to increase with age[2,4]. To test this hypothesis, experimental evidence was sought by examining second metaphase chromosomes of cultured oocytes of mice. In the previous experiment[5], abnormal segregation was induced in young mice, aged 3–6 months, by exposing them to a low dose of whole body gamma irradiation from a ^{137}Cs source. We have now found that radiosensitivity increases with age.

Virgin female (C$_3$H×ICR/Swiss)F$_1$ hybrids, similar to those used in the previous experiment, were irradiated at age 12 months and killed within a week of exposure. The same proceedure as before was followed for irradiating the females and handling controls. As before, there was no exposure to gonadotrophic hormones and the radiation dose (10–30 R) was kept low to minimise chromosome breakage. The details for obtaining second metaphase chromosome preparations have been described before[5]. Oocytes were placed on a slide and all were accounted for during the analyses. All slides were coded to avoid bias.

After analysis of the preparations by Giemsa staining, coverslips were removed by holding the slides under warm running tap water. The preparations were then air dried. C-banding, not used in the previous experiment, was used when the chromosome count was doubtful. The method of Arrighi and Hsu[6], with modifications suggested by C. E. Ford and E. P. Evans, was used. The dry slides were incubated at 70 °C overnight, placed in warm 2× SSC (0.3 M NaCl and 0.03 M Na citrate) and returned to the incubator for 90 min. They were then stained for 3–5 min in 1 : 20 Giemsa-phosphate buffer, *p*H 6.8, rinsed with fresh buffer and air dried. The best results were obtained when the slides were 1–2 weeks old.

Because of the age of the animals, 2.5 times as many females were required in the second experiment as in the first in order to obtain sufficient oocytes in second metaphase. There was an average of 11 eggs per mouse. Of the oocytes collected 39% proceeded into metaphase II (Table 1). Young females had an average of 36 eggs each, with 25% proceeding into metaphase II. The increased proportion of aged cells in metaphase II is highly significant ($P<0.0001$). On the other hand, significantly more oocytes of young mice remained in metaphase I ($P<0.0001$). Because the oocytes were teased out of the ovaries, these differences probably reflect variations in age of the cells collected. In young mice there is probably a wider distribution of oocytes between early and late dictyotene which could affect the timing of *in vitro* division. Moreover, as a mouse ages the less hardy oocytes may have degenerated *in vivo*.

Table 1 Frequencies of oocytes collected in first metaphase and second metaphase after 18–23 h *in vitro* culture

	Irradiated					Non-irradiated				
	Total	MI	MII	Other stages	Degenerated, no response	Total	MI	MII	Other stages	Degenerated, no response
Aged mice	5,957	430(7.2)	2,269(38.1)	25(0.4)	3,233(54.3)	6,207	399(6.4)	2,518(40.6)	36(0.6)	3,254(52.4)
Young mice	8,046	789(9.8)	2,064(25.7)	34(0.4)	5,159(64.1)	7,667	889(11.6)	1,866(24.3)	24(0.3)	4,888(63.8)
Total	14,003	1,219(8.7)	4,333(30.9)	59(0.4)	8,392(60.0)	13,874	1,288(9.3)	4,384(31.6)	60(0.4)	8,142(58.7)

MI, metaphase I; MII metaphase 2. Figures in parentheses are percentages.

Comparisons between the frequencies of hyperhaploid cells in young and aged mice are shown in Table 2. Hyperhaploidy occurred in irradiated samples at a significantly higher frequency than in controls. When ageing alone is considered, frequencies of nondisjunctional events increased with age, but the increases were not significant for either irradiated or non-irradiated mice. When these numbers are doubled as an estimate of the complementary hypohaploid cells, all comparisons are highly significant. The incidence of nondisjunction among aged controls was 0.6%, increasing to 2.7% on irradiation. The nondisjunction rate for irradiated young females was 1%.

Table 2 Frequencies of numerical aberrations in second meiotic metaphase oocytes of aged mice compared with young mice, irradiated and non-irradiated

		Irradiated (I)				Non-irradiated (C)			
		No. of oocytes analysable	No. of chromosomes			No. of oocytes analysable	No. of chromosomes		
	Dose (R)		19	20	21		19	20	21
Aged mice* (12 months)	10	390	11	370	7	411	16	390	1
	20	376	12	357	5	545	14	524	0
	30	351	18	321	3	350	15	330	3
	Totals	1,117	41	1,048	15	1,306	45	1,244	4
Young mice† (3–6 months)	Totals	1,149	34	1,109	6	1,054	31	1,023	0

For comparison of hyperhaploid cells only—young I against young C, $P = 0.02$; old C against young C, $P = 0.09$ (Fisher exact test); old I against old C, $P < 0.01$; all I against all C, $P < 0.001$; all I against young I, $P < 0.07$; χ^2 with Yates' correction for small numbers).

* Cells with chromatid breaks omitted.

† Details in ref. 1.

Table 3 Frequencies of chromatid breaks and separations

	Dose (R)	Irradiated			Non-irradiated		
		No. analysable	No. with breaks	No. with separations	No. analysable	No. with breaks	No. with separations
Aged mice	10	390	2	15	411	4	27
	20	376	2	25	545	7	21
	30	351	9	12	350	2	19
Totals		1,117	13	52	1,306	13	67
Young mice	10	426	0	2	379	0	5
	20	368	0	3	421	0	2
	30	355	0	11	254	0	2
Totals		1,149	0	16	1,054	0	9

The frequency of chromatid breaks in the oocytes exposed to 30 R (Table 3) was significantly higher than the total number of breaks found in cells exposed to lower doses ($P<0.01$) and in non-irradiated controls ($P<0.03$). Two of these cells had translocations (Fig. 1), and one other chromatid exchange occurred in a cell exposed to 20 R. There were no differences in breakage rates between the controls and cells exposed to 10 R or 20 R. In this sample the critical dose causing a substantial increase in chromatid breaks in the oocytes of aged mice seems to be higher than 20 R. It is difficult, however, to obtain accurate breakage frequencies in metaphase II cells because fragments can be lost during first anaphase. No chromatid breaks were identified in metaphase II chromatids of young females. The frequency of premature chromatid separation was increased in aged mice whether irradiated or not (Table 3). The primary purpose of this experiment, however, was to evaluate the effect of radiation on meiotic chromosome segregation.

Yamamoto *et al.*[7] found spontaneous and induced nondisjunction among the progeny of aged females but not of young mice. Lüning *et al.*[8] examined the effects of low dose X-ray exposure and age of females on the rate of intrauterine death, used as an indication of aneuploid offspring. They found that late maternal age at the time of mating had a considerable effect on foetal death rate but no detectable influence was noted from X-ray exposure with doses of 2–32 R. Reichert *et al.*[9] induced chromosome anomalies with X rays and found nondisjunctional products among second metaphase oocytes of irradiated females and none in controls: the difference, however, was not significant. They did not exclude the possibility that X rays induce abnormal segregation.

Our results with mouse oocyte chromosomes indicate that abnormal segregation may be induced during first meiotic division by *in vivo* exposure of mouse ovaries to low doses of radiation. This radiosensitivity seems to increase with age; the increase in abnormal segregation is more than twice the frequency found in young mice. This evidence in the mouse supports the suggestion that the risk of producing trisomic offspring among humans is increased with exposure of the abdomen to diagnostic X rays. Human radiation response has also been found to be related to late maternal age[3,4]. Examination of the results of 11 epidemiological studies[10] indicates that all but two[11,12] show an increase in nondisjunction with radiation exposure, although in some series these increases were not statistically significant.

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Photo-oxidation of water in phospholipid bilayer membranes containing chlorophyll *a*

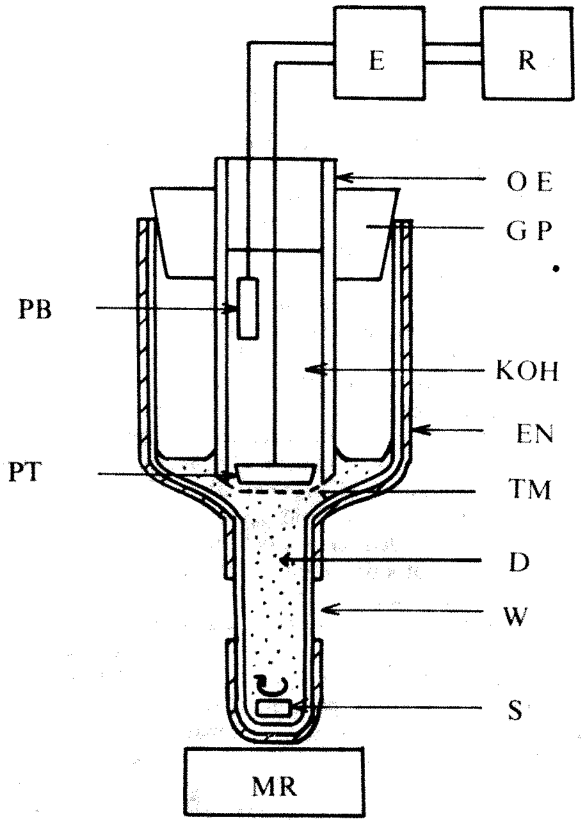
THE process of electron transfer from water to NAD in living photosynthetic systems involves a crystalline or liquid crystalline two-dimensional lattice of chlorophyll and other pigments. We describe here an artificial model system, with one of the characteristics of pigment system II, in which oxidation of water under the influence of red light (680 nm) is mediated by chlorophyll incorporated into bilayer membranes.

There have been several investigations of the photosensitivity of chlorophyll incorporated into phospholipid bilayers. For example, when chloroplast extracts were incorporated into the phospholipid bilayer separating two aqueous solutions of different redox potential, photocurrents through the bilayer were observed[1-3]. A peak was observed at 680 nm, corresponding to the red absorption of chlorophyll *a*[4]. Nicholls *et al.* incorporated chlorophyll a into lipid liposomes and observed the photooxidation of added ferrocytochrome *c*[5], and Tomkiewicz *et al.* observed photoproduction of the chlorophyll *a* cation radical in the presence of the water-soluble oxidants[6]. This suggests that redox reactions take place at the membrane–solution interface under the influence of light absorption by chlorophyll *a*. We report here light-induced oxidation of water in aqueous dispersions of phospholipid liposomes containing chlorophyll *a* and *b* together with small amounts of other pigments within the chloroplasts of spinach leaves.

To confirm that photo-induced oxygen production takes place at one surface of the bilayer membrane and is associated with a redox reaction at the other surface, we used liposome dispersion systems containing water-soluble oxidants only in the aqueous compartment inside each liposome.

A preparation consisting of chlorophyll *a* (68.0 weight %) and *b* (22.8 weight %), and pigments such as carotene and xanthophyll (9.2 weight %) was prepared from spinach leaves by repeated precipitation in a methanol–dioxane–water mixture in the appropriate proportions[7]. Phosphatidylcholine was isolated chromatographically from egg yolk[8]. Liposome dispersions were prepared by suspending colyophilised phosphatidylcholine (100 mg) with a given amount of the chlorophyll preparation and a trace of carbonylcyanide D–trifluoromethoxyphenyl hydrazone (FCCP) in a 5-ml aqueous solution of appropriate composition. The suspension was irradiated ultrasonically with a sonicator at 20 kHz and 4 °C under argon for 15 min. Undispersed phospholipids were removed by centrifugation at 13,000*g* for 10 min. The resulting supernatant was subjected to molecular sieve chromatography at 4 °C on a column of Sepharose 4 B. The elution profile of the dispersion consisted of two distinct peaks. The position of the second peak was independent of the molar ratio of chlorophyll *a* to phosphatidylcholine in our experimental conditions and was similar to that observed for liposome dispersion without chlorophyll. Thus the liposomes in this peak were about 250 Å in diameter[9]. To prepare a system in which oxidants existed only in the aqueous interior of each liposome, sonication was carried out in an aqueous solution of 0.1 M potassium ferricyanide, 0.5 M Tris-chloride and 0.1 M potassium chloride at *p*H 7.50. $K_3Fe(CN)_6$ was removed from the external aqueous phase by rapid passage of the liposome dispersion at 4 °C over a column of Sephadex G-50 which had been equilibrated with the aqueous solution of 0.5 M Tris-chloride and 0.1 M KCl at *p*H 7.50. A sample of 5 ml of the resulting dispersion was transferred to a glass cell with an oxygen electrode connected to a high speed electrometer (417 High Speed Picoammeter, Keithley, Fig. 1). The outside of the cell was coated with a thin layer of black enamel to protect it from stray light, except for a window of 1.2 cm² through which the liposome system was irradiated with light. In particular the oxygen electrode was not exposed to light. Before irradiation with light, the dispersion was bubbled with Ar gas for 20 min to remove dissolved oxygen. A 500-W xenon lamp with an infrared cut off glass filter was used throughout the experiments and light intensity was varied by means of neutral density filters. A monochromator or a set of interference filters was used as needed.

Fig. 1 Schematic diagram of experimental setup for measurement of light-induced oxygen production in the chlorophyll-liposome system. E, electrometer; R, recorder; GP, gum plug; OE, oxygen electrode; KOH, 0.1 M KOH solution; PB, Pb electrode; PT, Pt electrode; TM, Teflon membrane; EN, black enamel layer; D, dispersion of liposomes; W, window; S, stirring bar; MR, rotary magnet.



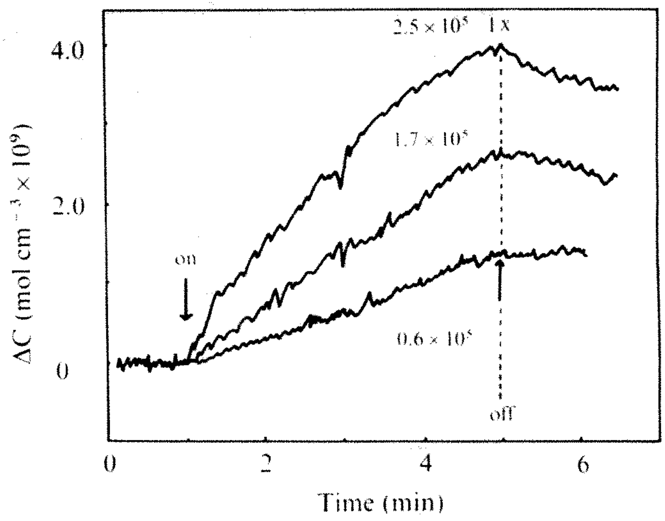
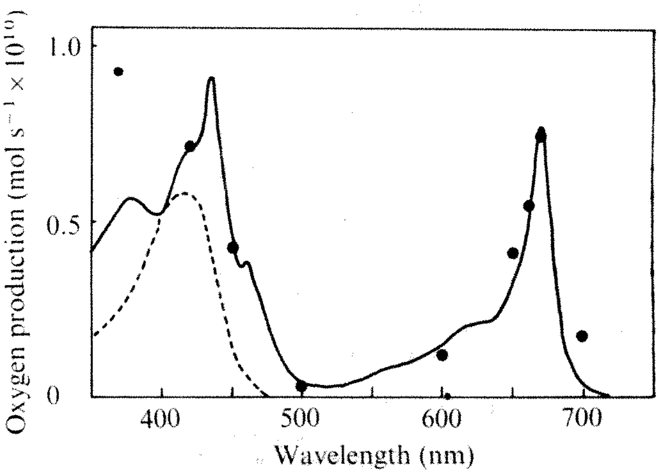


Fig. 2 Change of oxygen concentration in the liposome dispersion when illuminated by white light of three different intensities. Numbers represent light intensity in lx.

Figure 2 illustrates the change in oxygen concentration in the external aqueous phase under white light illumination at various intensities. The data were obtained in a dispersion of the liposomes composed of 100 mg of phosphatidylcholine and 1.0 mg of chlorophyll preparation. The rate of oxygen production calculated from the initial slope of each line was nearly proportional to the light intensity. The rate of oxygen production at 100,000 lx was 4.2×10^{-11} mol^{-1}. Figure 3 compared the action spectrum of the photoproduction of oxygen in the same system with the absorption spectra of chlorophyll incorporated in the liposome and of $K_3Fe(CN)_6$ in aqueous solution. The position of the red band of chlorophyll *a* in the liposome bilayer was similar to that observed for chlorophyll *a* in ethanol solution. As Fig. 3 shows,

Fig. 3 Comparisons of the action spectrum for oxygen production with the absorption spectra of the chlorophyll sample in the liposome bilayer and of $K_3Fe(CN)_6$ in aqueous solution. ●, Action spectrum for oxygen production; ——, absorption spectrum of the chlorophyll sample in the liposome bilayer; – – –, absorption spectrum of $K_3Fe(CN)_6$ in aqueous solution.



the action spectrum has two peaks around 420 nm and 670 nm, corresponding to the absorption bands of chlorophyll *a* in the liposome bilayer, but has no similarity to the absorption spectrum of $K_3Fe(CN)_6$. These results suggest that the reaction proceeds by utilising photoenergy absorbed by chlorophyll. In the absence of $K_3Fe(CN)_6$ in the compartment of each liposome, oxygen production was scarcely observed even in the light.

Tomkiewicz *et al.* observed the photoproduction of the chlorophyll cation radical in the dispersion of liposomes containing chlorophyll a in the presence but not in the absence of $K_3Fe(CN)_6$ (ref. 6). Taking their results into consideration ours might be expressed

$$\text{chl} + h\nu \longrightarrow \text{chl}^* \quad \text{photo-excitation} \qquad (1)$$

$$\text{chl}_i^* + \text{Fe}^{3+} \longrightarrow \text{chl}_i^+ + \text{Fe}^{2+} \quad \text{chl cation radical formation} \qquad (2)$$

$$\text{chl}_i^+ + \text{Fe}^{2+} \longrightarrow \text{chl}_i + \text{Fe}^{3+} \quad \text{overall back reaction} \qquad (3)$$

$$\text{chl}_i^+ + \text{chl}_o^* \text{ (or chl}_o) \longrightarrow \text{chl}_i + \text{chl}_o^+ \quad \text{charge transfer} \qquad (4)$$

$$\text{chl}_o^+ + 1/2\,\text{H}_2\text{O} \longrightarrow \text{chl}_o + 1/4\,\text{O}_2 + \text{H}^+ \quad \text{oxygen production} \qquad (5)$$

In these equations, chl* and chl^+ are excited chlorophyll *a* and chlorophyll *a* cation radical, respectively, and Fe^{3+} and Fe^{2+} represent ferricyanide and ferrocyanide ions. Chl_i and chl_o are the chlorophyll *a* molecules localised in membrane surfaces facing the aqueous phase inside each liposome and the ambient aqueous phase outside the liposome, respectively.

The explanation of these equations is as follows. Chlorophyll *a* is excited by light at both membrane–solution interfaces (equation (1)), but chl^+ is produced only inside the liposomes (equation (2)). The back reaction may occur at the internal surface (equation (3)). Competing with this back reaction, charge exchange between chl_i^+ and chl_o^* (or chl_o) might take place through a redox species present in the membrane, such as carotene and xanthophyll[10] (equation (4)). The chl_o^+ thus generated in the outer surface may oxidise water in the external aqueous phase; consequently oxygen is produced in the ambient aqueous phase. The excess negative charge accumulated inside each liposome by the charge exchange between chl_i^+ and chl_o^* (or chl_o) may be compensated by FCCP-mediated proton transport from the external to the internal aqueous solution of the liposomes.

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Structure of nosiheptide, a polythiazole-containing antibiotic

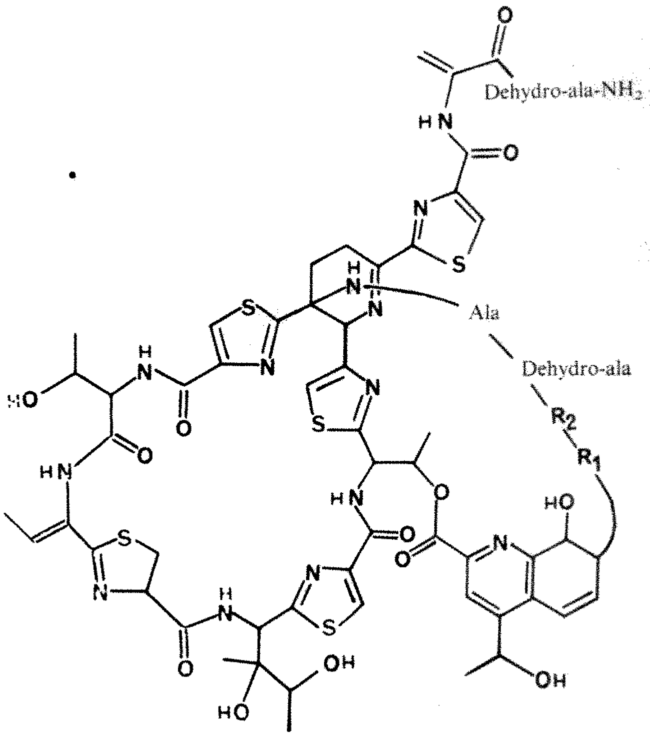
NOSIHEPTIDE is a metabolite with a strong antibiotic action which has been isolated from *Streptomyces actuosus* (F. Benazet *et al.*, unpublished). Studies of its antibiotic spectrum and cross-resistance suggested that it belonged to the thiostrepton family[1]; chemical analyses and further chemical degradation confirmed this view. From analytical and spectroscopic data (NMR ^1H, ^{13}C and ^{15}N) the general formula $C_{51}H_{43}N_{13}O_{12}S_6$ was assigned (A. Brun, H. Depaire, G. Lukacs and J. P. Thomas, unpublished). Unfortunately no M^+ peak can be extracted from its mass spectrum (electron impact). Some parts of the molecule have been identified after degradation by different techniques, namely one L-threonine, one hydroxypyridine, five thiazoles and one indole ring (A. Brun, H. Depaire, G. Lukacs, and J. P. Thomas, unpublished). Nothing could be deduced about the sixth sulphur atom. At least twenty antibiotics are known to contain thiazole rings (for instance bleomycins[2], bacitracin A[2], and althiomycin[3]), but among those containing five thiazole rings only two molecular structures are known: thiostrepton, by X-ray analysis[4] and ^{13}C NMR spectroscopy, and siomycin A, by comparing its NMR spectrum with that of thiostrepton[5]. To obtain the complete structure of nosiheptide, we undertook an X-ray analysis.

Crystals of nosiheptide were grown in a mixture of $CHCl_3$ and C_2H_5OH (3:1) by slow evaporation at 30 °C. Any attempt to change the ratio of the two solvents led to powdery precipitates or unusable small crystals. They were mounted in Lindemann glass capillaries in the presence of solvent which is part of the overall crystal structure, as these crystals decompose as soon as the mother liquor is removed. Crystal data: $C_{51}H_{43}N_{13}O_{12}S_6$, molecular weight = 1,222, tetragonal, space group $I4_1$; $a = b = 36.05$, $c = 11.44$ Å, $Z = 8$ and $V = 14{,}735$ Å^3. (It is noteworthy that thiostrepton, with a similar molecular structure, was first found in the tetragonal system before it was crystallised in the monoclinic form.) A rapid decrease in intensities was found at higher diffraction angles and only 2,740 reflections (spacing limit θ up to 60°) could be collected by a computer-controlled diffractometer using CuKα radiation (filtered by a graphite monochromator) in the $\theta/2\theta$ scanning mode. Intensities were corrected for the effects of slow decomposition and by the Lorentz and polarization factors but not for absorption.

The measured density is 1.41 g cm^{-3}, as in thiostrepton. The calculated density, with eight molecules in the cell, is 1.107 g cm^{-3}. Consequently there is a difference of 336 in total mass of the asymmetric unit which is attributable to the solvent.

Attempts to find the trial structure by the sharpened Patterson method were unsuccessful. The structure was solved with some difficulty by the combined use of the phase function[6]

Fig. 1 Condensed formula for thiostrepton (R_1–R_2 = Ile–Ala) and siomycin A (R_1–R_2 = Val–Dehydro-ala). (From ref. 5.)



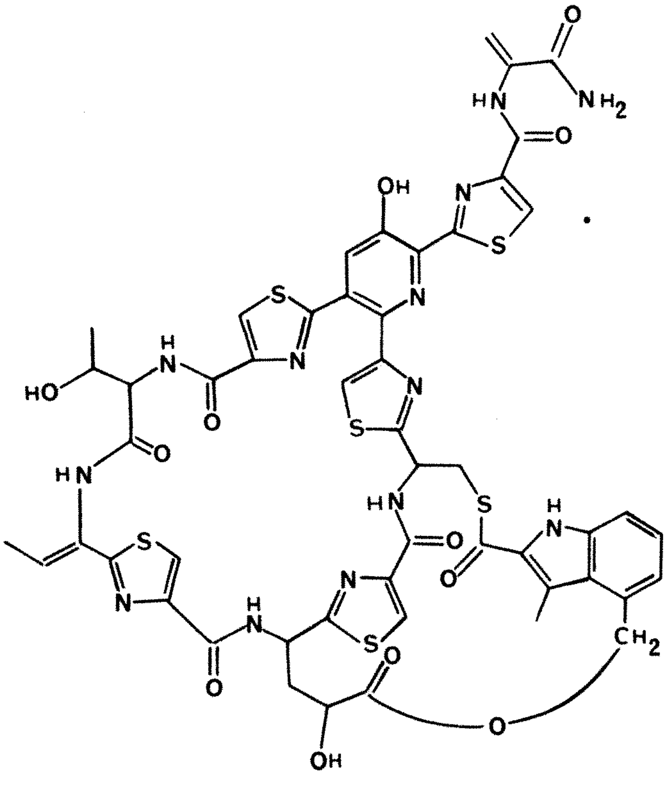
(to select the starting set) and the multisolution method[7] (for tangent refinement). Starting with the four highest peaks of the best E map, regarded as sulphur atoms, a long carbon chain was revealed. A second cycle led to the location of the pyridine ring and the three bonded thiazole rings. Further recycling revealed the complete structure. Identification of the different atoms was made on the basis of chemical evidence (A. Brun, H. Depaire, G. Lukacs and J. P. Thomas, unpublished), temperature factors, bond distances and angles. Details of the crystallographic problems encountered will be published later.

Full matrix least-squares refinement with isotropic temperature factors gave a conventional residual *R* of 25%. The sulphur atoms alone were then assumed to be anisotropically vibrating. Because of the small number of reflections, the rest of the molecule was kept isotropic. The final *R* was 18% with a Cruickshank[8] weighting scheme.

No significant peaks could be recognised in the last difference Fourier synthesis (maximum peak 1 e Å^{-3}) except weak peaks attributed to one $CHCl_3$ molecule with a low occupation factor. The rest of the solvent was therefore assumed to be randomly distributed.

The formula of nosiheptide is shown in Fig. 2 and may be compared with that of thiostrepton (Fig. 1). Some interesting differences are seen: (1) The tetrahydro pyridine cycle (anchorage part of the molecular chains) as well as the thiazoline ring of the thiostrepton backbone, are unsaturated in nosiheptide. Consequently the three chains can be linked to each other in a more planar conformation than the four chains of thiostrepton. Following this observation, the upper part of the molecule is conjugated to the pyridine ring, allowing it to be nearly coplanar, and therefore is much less agitated than the proposed side chain of thiostrepton. The presence of a free hydroxyl group on the pyridine ring might be used to prepare chemical derivatives for further biochemical studies. (2) The quinaldic precursor (quinoline derivative) is replaced by a methylindolic compound of probable similar biosynthetic origin, but differently bridged over the chains. (3) The sixth sulphur atom is found in an unusual thiol-ester linkage. This was rather unexpected on the basis of initial thiostrepton analogies and is, to our knowledge, the first thiol-ester function isolated in an antibiotic compound.

Fig. 2 Nosiheptide, general formula. Chains are attached together by three asymmetric centres which all have the S configuration, deduced from the L-threonine residue.



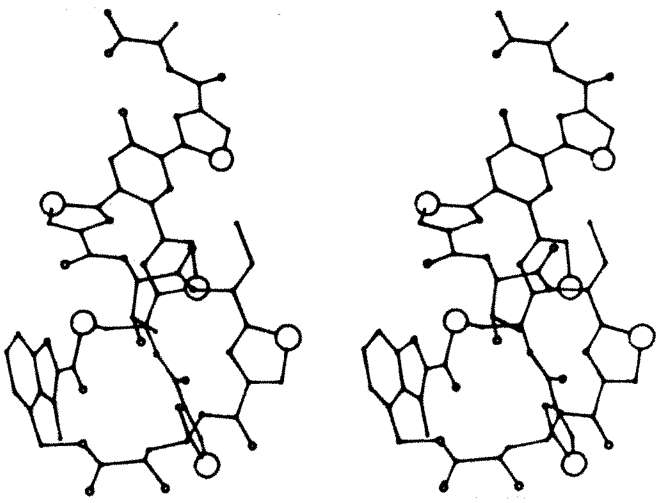


Fig. 3 Stereoscopic view of nosiheptide. The general globular conformation of thiostrepton is very similar, despite the absence of the fourth chain. Drawings of the molecular packing of this structure reveal large void channels in which solvent is in complete disorder.

A stereoscopic view of the molecule is given in Fig. 3 and clearly shows the linkage of the indolic part over the macrocycle constituted by thiazole rings.

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Erratum

In the article 'Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells' by Richard J. Pietras and Clara M. Szego (*Nature*, **265**, 71; 1977), the ordinate labels to Fig. 3 should read from 0 to 80, not from 10 to 90.

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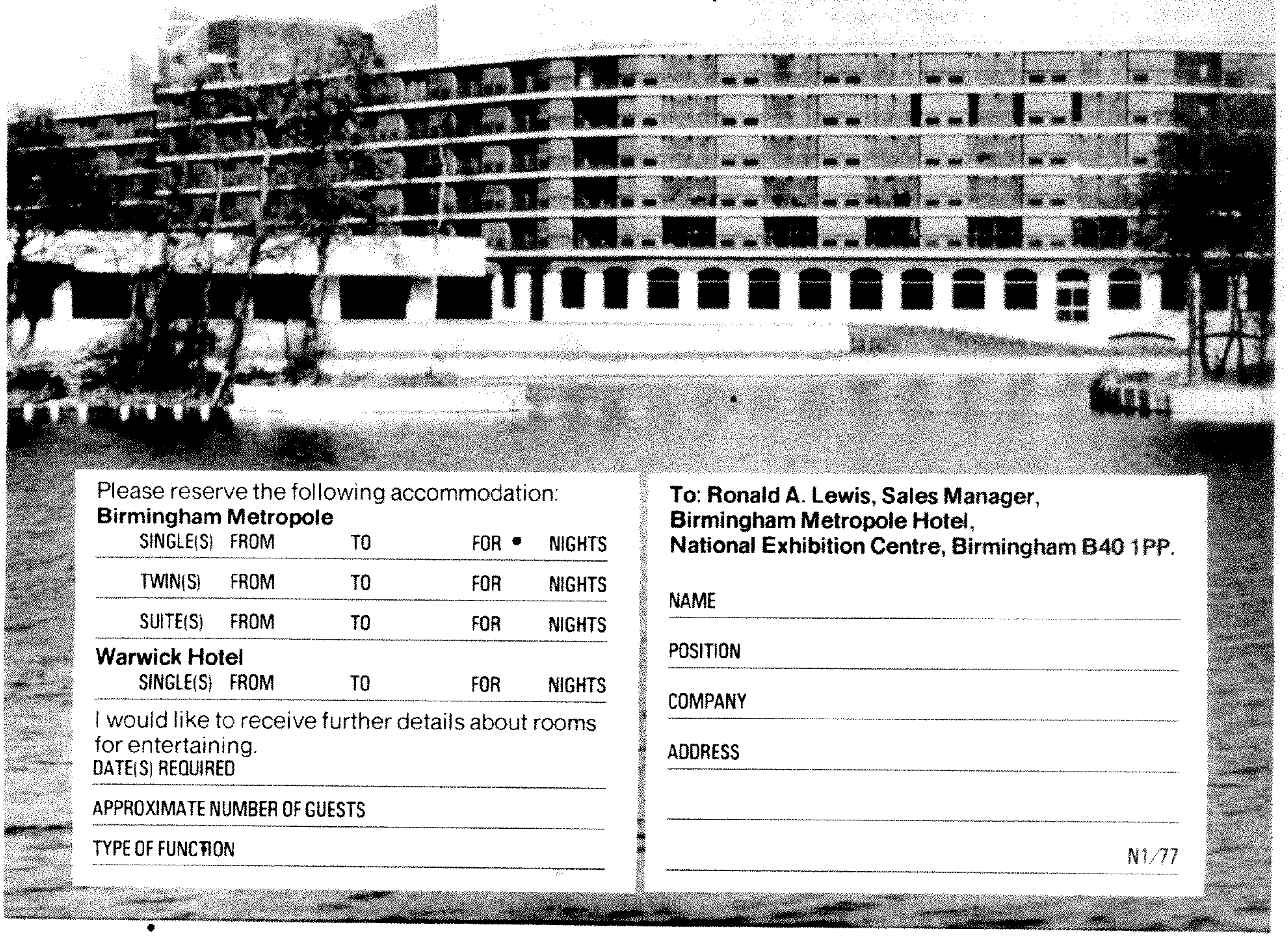

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reviews

Institutionalised science in America

J. B. Morrell

The Pursuit of Knowledge in the Early American Republic: American Scientific and Learned Societies from Colonial Times to the Civil War. Edited by A. Oleson and S. C. Brown. Pp. xxv+[illegible] (Johns Hopkins: Baltimore and London, July 1976.) £11.55.

In 1769 the American Philosophical Society (Philadelphia), the oldest learned society in the USA, was reorganised into its present form under the presidency of Franklin, who had originally founded it in 1743. Less than a century later, America had produced two national scientific societies: the voluntary American Association for the Advancement of Science (AAAS, founded 1848), and the federally sponsored National Academy of Sciences (founded 1863). After the Civil War both local and national scientific societies were eventually replaced by universities, federal bureaux, specialist societies, and private foundations, as the principal means of discovering, certifying, and diffusing scientific knowledge. Yet in the first hundred years of the American republic, the learned society, devoted to general science, *belles lettres*, technical instruction, agricultural improvement, or the promotion of manufactures, constituted the chief genre of organisation through which knowledge was pursued.

The eighteen papers in this volume were commissioned by the American Academy of Arts and Sciences as the first phase of a collaborative study of the history of institutionalised science and learning in America. By focusing on the scientific centres of the eastern states, such as Philadelphia, Boston, New York and Albany, several contributors confirm the importance of regional differences. The historical significance of local context is, however, best revealed by Shapiro's lively analysis of the Western Academy of Sciences at Cincinnati, the Porkopolis of the mid-West. The theme of scientific knowledge as public utility, conceived patriotically and democratically, is also well covered. Indeed almost all the contributions, which are adequately indexed, deal competently with the staple features of institutional history; and they bristle with dedicated documentation.

By the end of the nineteenth century American science had become 'professional' in several ways: arcane specialisation was rampant; training procedures in laboratories and lecture rooms were well established; qualifications of scientific competence were publicly available and recognised; and many full-time paid jobs in science were filled by certificated individuals. Quite naturally several contributors have been enticed by the theme of professionalisation, the obvious example being Kohlstedt in her account of the ways in which the AAAS reflected and shaped the allegedly professional pattern of the 1850s.

Other authors, however, will have none of this. In a forthright essay Reingold stresses the social and intellectual inclusiveness of most *ante bellum* scientific societies, and accordingly he offers a speculative taxonomy of the scientific community divided into researchers, practitioners, and cultivators. Shapiro's essay erodes the professionalisation model even further by rejecting as unpardonably anachronistic any attempt to divide the membership of American scientific societies into distinct occupations before about 1850.

Irrespective of their attitude to whether professionalisation is a valid organising theme, most contributors present overwhelming evidence to show that many societies failed to fulfil their own stated purposes and that some declined into paralysed, helpless, and hopeless organisations. Especially in a democratic country, where the privilege of learned societies was sometimes suspect, their establishment, maintainance, and growth required the persistent attention of devoted individuals who saved them from precarious existence or quiet extinction. When the continuing existence of so many societies was a pervasive problem, one feels that the professionalisation model is an inappropriate key to their history. Rather one suspects that the origins, purposes, and features of these societies may be interpreted in terms of the various interests, ideas, and resources of the participating and sometimes conflicting members. At least, one would expect that scientific societies faced difficulty in translating aims into action.

For these reasons the most interesting essays are those which deal with failure or which give considerable weight to the aims and perceptions of individual scientists. Hindle, for instance, accounts for the failure of New Yorkers to establish a viable scientific society rivalling those of Philadelphia and Boston by arguing that they preferred to expend their energies in various societies for practical arts modelled on the London Society of Art and not the Royal Society.

Gerstner's analysis of the changing features of the Philadelphia Academy of Natural Sciences reveals the dominance from 1817 to 1840 of William Maclure and the subsequent challenge to it. Sinclair gives similar coherence to his study of the Franklin Institute of Philadelphia by showing how its multifarious activities of the 1820s were redirected by Alexander Dallas Bache into experimental technical research, publication, and public funding. Starting from the assumption that scientific societies only exist when they serve the needs of members as well as those of science, Shapiro shows that in the Cincinnati Academy the unresolved conflicts about classification led to its evanescence in 1854.

Although Kohlstedt employs the professionalisation model, her results reveal the persistent feuding within the early AAAS between the advocates of social inclusiveness led by Henry Darwin Rogers and the elite group of Lazzaroni committed to exclusiveness. Not surprisingly the latter led by Bache became dis-satisfied with the AAAS, and found the highly select National Academy of Sciences far more congenial.

All these contributions confirm that during the past ten years or so American historians have consolidated the history of American science as a lively field, distinguished generally by competence and occasionally by *éclat*. This volume is an admirable testament to that enviable achievement. ☐

J. B. Morrell is a Lecturer in the History of Science at the University of Bradford, UK.

Problems of quantum biochemistry

Quantum Biochemistry and Specific Interactions. By Z. Simon. Pp. 251. (Abacus: Tunbridge Wells, Kent, UK, 1976.) £11.95.

APPLYING the methods of quantum chemistry to problems in biochemistry is so fraught with difficulties that high levels of scepticism are required in addition to enthusiasm for the subject. Until very recently theoretical chemists were not notably successful in calculating properties of small isolated molecules let alone large molecules in aqueous solution or in even more complicated environments.

Over the past few years the problems of molecular physics have become tractable due to refinements of molecular orbital techniques and the power of computers. With the confidence gained from success with the isolated molecule, theoreticians are becoming more interested in biological problems; in particular conformational studies and comparisons of reactivity are proving valuable. Professor Simon's book, originally published in Romanian in 1973, was written before the advances of the past few years, but must have been almost out of date even then.

The molecular orbital technique described in the chapter which follows the introduction is almost totally devoted to π-electron Hückel theory; a final section on 'advanced methods' lists variants more likely to be referred to as 'crude' in current publications; and neither *ab initio* nor PCILO approaches are mentioned. None of the many references cited is later than 1973 despite the fact that the work is described as a 'revised, up-dated translation'. It is an elaboration of the work of the Pullmans in their 1963 book on quantum biochemistry rather than the total revision which would be much welcomed.

Similarly, the chapter on intermolecular forces rests heavily on work by the Pullmans and Scheraga, which both groups would probably consider to have been superseded. Reactivities are discussed in terms of parameters deduced from π-electron calculations rather than the potential surfaces currently in vogue.

The specific interactions referred to in the title and an analysis of some recognition processes occupy the last two chapters of the book. Enzyme reactions, repressor-operator interactions and specificity in immune phenomena are all considered, but much more space is devoted to descriptions of the topics and to quantitative measures than to applications of quantum mechanical methods.

The strength of the book is its collection of facts and topics of biochemistry which remain as problems rather than a description of how they have been solved. The descriptive portion like the theory suffers from the time lag in appearance of the work, but it is impossible to read without feeling an urge to tackle some of the problems with more up-to-date methods.

The book is in no way an account of the state of the art but it is a valuable collection of topics and references more to be recommended to the seasoned campaigner than to the student.

Graham Richards

Graham Richards is a Lecturer in the Department of Physical Chemistry at the University of Oxford, UK.

Whirlpool of aggression

The Evolution and Chemistry of Aggression. By D. D. Thiessen. Pp. xviii+211. (Charles C. Thomas: Springfield, Illinois, 1976.)

THE first chapter of this book is entitled "The Evolution of Competitive Behavior". The other four are on gene organisation, endocrine control, chemical social signals, and the actions of drugs. There is a bibliography of about 300 entries, and a very brief subject index. The author's stated intention is to investigate aggression in relation to evolution and chemistry. "Unless otherwise noted", he writes, "aggression will refer to intermale aggression."

I read the first chapter in some bewilderment. "Aggression," we are told, "in one form or another, is as old as life itself." The first pages include passages on 'molecular aggression', competition between species, predator–prey interactions, and species diversification. There follows a section on "a move toward intraspecific competition" devoted largely to the unusual conduct of a species of sea anemone. There is then one on brain differentiation, in which there are references to 'competitive readjustment of brain size' and 'selection for neurological competence to deal with distance perception'.

Dr Thiessen evidently equates aggression with competition. "If an animal fails to fight", he tells us, "we too readily conclude that he is non-aggressive. . . . Even an animal that does not fight . . . may still be highly competitive." In some passages competition seems to be thought of as equivalent to what goes on in the private sector. The biological principles that deal with competition are those of population genetics, but they are disregarded. Instead, we have tautologies: for example, "there is positive selection for those forms that optimize their use of space and commodities and negative selection for those forms that fail." Similarly, none of the recent critical reviews on natural selection in relation to behaviour, or on intolerant behaviour within species, are represented in the bibliography.

Dr Thiessen's chapter on his own speciality, the endocrine control of behaviour, summarises much interesting work, but again without a unifying theme. Among the many topics mentioned are sex reversal in fish and mammals, the disorganisation of sexual development by early treatment with hormones, the metabolism of the hypothalamus, hormones and social status, and human chromosome abnormalities. There is also a discussion of the behavioural changes that accompany the human menstrual cycle.

Dr Thiessen is not always uncritical. In this field the danger of inferring causal relationships from correlations is always with us. For example, violent prisoners have high plasma testosterone levels. Dr Thiessen remarks that the violence may not be due to the hormone: instead imprisonment could increase testosterone secretion. And on page 82 he utters an important warning: "specialised definitions of aggression involving violence and sexual assault may be more amenable to investigation than more generalised notions of aggression."

This principle could have been usefully applied to the chapter on drugs. "A survey of the literature", we learn, "indicates that almost all classes of drugs studied have been found to decrease, increase or not influence aggression." This is not surprising when we find, a few pages later, a list which includes "footshock aggression" (probably defensive postures induced by pain), "isolation aggression" (possibly a response to strangeness), "irritable aggression" due to brain injury, "aggression" induced by various drugs, and predatory behaviour.

The editor of the American Lecture Series, Dr I. N. Kugelmass, writes in his foreword: "What is aggression? It is life itself—a whirlpool in many ways. When once set agoing it spins on and on." As Australians say, too right.

S. A. Barnett

S. A. Barnett is Professor of Zoology at the Australian National University, Canberra.

obituary

Luigi Gorini, who was a professor emeritus of microbiology and molecular genetics at Harvard Medical School died on August 13 at the age of 72 in Boston. Luigi was born in Milan, in 1903. Although he received his scientific training in the 1920s, he did not really enter his research career until after the second World War. Because he was one of the few scientists who refused to pledge an oath to the fascist government of Italy in the 1930s, he was unable to secure academic employment. He eventually joined the anti-fascist underground.

At the end of the war in Italy, Luigi was a prime mover in establishing a refuge for the many children who were orphaned. Restricted by lack of money and facilities, Luigi decided that the most needy children were Jewish orphans and he set up and ran the home at Selvino for them prior to their emigration to Israel. In 1976, the account of these activities by Luigi and his wife Annamaria Torriani was laid in the Martyrs and Heroes Archives at Yad-Vashem, Israel.

His first scientific paper was published in 1946 when he was 42 years old. Much of Luigi's early work was on the mechanism of protection of various bacterial proteases by ions such as calcium and manganese. He and his coworkers were able to show that the metal ions protected these enzymes against autodigestion by stabilising particular protein conformations. This work had wide impact in that it provided a strong suggestion that proteins do not have unique folding patterns, but can exist in several different stable states.

Upon coming to the United States, Luigi's interests shifted to problems of gene regulation in *Escherichia coli*. In collaboration with Werner Maas at New York University, he used the chemostat to study steady state growth of several arginine auxotrophs with and without arginine. In this way they discovered a large derepression effect on the arginine pathway and were able to show elegantly how repression and feedback inhibition could interact to exert a coarse and fine tuning effect on the rate of enzyme synthesis and arginine formation in that pathway. These studies helped to establish the concept of end-product repression and derepression in biosynthetic pathways. Luigi and his coworkers later continued to contribute to the understanding of regulation of arginine biosynthesis.

After moving to Harvard in 1957, Luigi's research interests expanded to a study of the effect of streptomycin on the translation of messenger RNA into protein. By demonstrating that streptomycin and mutations of the ribosome to streptomycin resistance could influence the accuracy of translation, Gorini and Kataja provided, in 1964, the first evidence that ribosomes were not merely passive templates for protein synthesis. Rather, ribosomes played an important role in determining the interaction between transfer RNA and messenger RNA. These proposals were confirmed directly by *in vitro* experiments in which it was shown that ribosomes sensitive to streptomycin promote mistakes in reading of the code in the presence of streptomycin and other aminoglycoside antibiotics. Subsequently, in 1969, Rosset and Gorini showed that it was possible to increase misreading by the ribosomes through altering a ribosomal protein by the *ram* (ribosomal ambiguity) mutation.

Thus again this large body of work brought Gorini's focus directly on to a basic mechanism for the control of all growth—this time at the level of translation of the gene code. His subsequent work centred around two of the components at this step—the ribosome and transfer RNA. Many of his results in this area were presented in a review on Informational Suppression in 1970 (*A. Rev. Genet.*, **4**, 107–134). His last projects were concerned with establishing an active role for streptomycin with the ribosome or on one of its components, and on the possible interaction of ribosomes in the coupled transcription–translating system.

In 1949, Luigi was awarded the Kronauer Prize by the Faculty of Science at the Sorbonne, and he won the Ledlie Prize of Harvard University in 1965. He was elected a member of the National Academy of Sciences in 1971.

Luigi remained active in his laboratory until the last few months before his death, both advising people in the laboratory and doing experiments himself. One remarkable feature of his scientific life is that, between the time of his official retirement age of 65 at Harvard Medical School and his death, he published 33 papers.

Luigi also remained active politically. He had many letters published in the newspapers protesting policies he opposed. When Henry Kissinger was awarded the Nobel Prize for Peace in 1973, Luigi organised a petition protesting this award. The petition was sent to the Nobel Committee and received publicity in the United States. He also was very active in the anti-Vietnam War movement and spoke out constantly against the use of genetic arguments to support racial discrimination. The following excerpt from a speech he gave in 1970 at Montana State University gives an idea of his conception of the political role of scientists:

> "My job here tonight is to make you realise that for me, like for hundreds of us scientists, my own scientific interest means a lot intellectually but, morally speaking, science alone does not satisfy entirely my conscience. I will try to be the most unequivocal radical possible and at the same time constructive, so that when I quit, your opinion about me should not be similar to that expressed a long time ago by the fascist Italian police about someone whom I know after his first confrontation with them. He was very happy to be released, for a time at least, but a few years later he discovered by chance the written motivation for letting him out and he was really not satisfied. The police file sounds like the following: 'Lonely anarchist; he is not dangerous'."

The following are excerpts from a conversation between the two of us, J. B. who worked in the same department as Luigi, and M. D. who worked in his laboratory.

M.D. Politics probably shared equal prominence in his life with science.

J.B. He would be horrified when people would do things that didn't fit in with his sense of morality. He never lost his sense of outrage.

M.D. Things were black or white to him. In a way it was a great strength of character. He would never compromise.

J.B. Very frequently in the morning he would come rushing down the hall into my office, holding a newspaper or asking me if I'd seen or heard the news that morning. We had to do something right away about it—compose a letter . . .

M.D. . . . or send off a telegram.

J.B. Or sometimes when he got home he had heard something on the news and he was so upset that he would have to call people. I connect his continued sense of outrage about anything political that upset him with his intense enthusiasm and involvement in his scientific work. He never mellowed in that sense.

M.D. He was always in the lab. before anyone else. He was so enthusiastic that he would have got everyone's plates out of the incubator before they came in.

J.B. It seems amazing to me that he maintained that involvement until the end—that it never dissipated.

M.D. He was talking once about a scientist who was retiring and was going to retire to live in Europe. He said "How can a man live like that without science? If I didn't have something to look forward to every day, what would my life be?".

He was not encouraging in that he would say: "That was a really nice experiment". But it was his enthusiasm that was so overwhelming. He tried to have a personal relationship with everyone in the lab. And discussions about projects, although sometimes heated, were usually fruitful, amicable, encouraging and often hilarious.

J.B. He was physically very active. A few years ago he was climbing mountains in the Dominican Republic.

M.D. We went to Mount Washington once, we drove to the top. We climbed down a way and then climbed back up to the car. He was really amazing. He was walking at a good pace, while everyone else was struggling up.

J.B. He was always telling jokes or funny stories. When he entered this country as an immigrant, he had to answer some questions. One of the questions was about adultery. Luigi answered the interviewer by asking "Do you want a yes or no answer or number of times".

M.D. He was such a good teacher to people in the lab. about life and about everything.

Jon Beckwith
Margaret Duncan

John Donald Rose, FRS, who died on October 14 was a former Research and Development Director of Imperial Chemical Industries Ltd. He was born on January 2, 1911 in Rotherham, near Sheffield and went to Rotherham Grammar School before reading chemistry at Jesus College, Oxford. Throughout his life, he never lost a certain bluff "no nonsense" Yorkshire earthiness. He was also a man of quite exceptional kindness and generosity of spirit, and had a well-developed sense of the ludicrous. The combination of these qualities was exceedingly attractive and it was well-nigh impossible not to like "J.D."

He took a First in chemistry at Oxford, and was awarded a Salters' Institute Fellowship, part of which he chose to spend working in Prof. L. Ruzicka's laboratory at the Eidgenössische Technische Hochschule in Zurich. He joined the Research Department of the Dyestuffs Division of ICI at Blackley in 1935 and is believed to have been the first person to make a sample of Nylon 66 in this country, just as he was later to be one of the first ICI men to develop Whinfield's recently discovered polyethylene terephthalate ("Terylene"). During the war he was a member of, and later head of an Exploratory Research Section, and carried out a thorough investigation of the chemistry of the nitroparaffins. At the end of the war, he was deeply involved in the detailed examination of unpublished German war-time research, and his good working knowledge of German made him a valuable interrogator. Characteristically he was kind and generous to German scientists who were being held as possible war criminals. Thereafter, he was rapidly promoted, becoming Research Director of the Dyestuffs Division in 1951.

It was during his term as Research Director that Rattee and Stevens discovered the reactive cotton dyes, the "Procions." J.D. knew very well that the ICI patent position and technological lead were not initially very strong, and he showed great courage in deploying a major effort, fully supported by his colleagues on the Development side, to get the "Procions" on to the market very quickly—in fact within two years. Considerable faith was needed to persuade the dyeing industry to adopt totally new techniques and concepts, but the venture succeeded and the first three dyestuffs were soon expanded into a full range.

In 1959 he was transferred to the Paints Division as joint Managing Director, becoming Chairman in 1964. In 1966 he joined the ICI Board as member responsible for research and development. He was elected Fellow of the Royal Society in 1971 in recognition of "his contributions to chemical science and its utilisation in industry." After his retirement in 1972 he held office as Master of the Salters' Company (1973), an honour which gave him particular pleasure.

It would be wrong to suggest that J.D. was an outstandingly original scientist—he never claimed to be one. His talent was a great clearness of mind, sound judgement and the ability to guide and encourage those associated with him. Many ICI scientists would gladly agree that their own developments or discoveries owe something, often far from negligible, to him.

He died at the age of 65, leaving a widow, son and daughter, and four grandchildren.

M. A. T. Rogers

Dr Alfred E. Emerson, the world famous termitologist died on Sunday October 3, 1976 at Glenn Falls, New York.

He was born in Ithaca and was educated there, at Cornell University. In 1922 he became an Associate Professor in the Department of Zoology at Pittsburgh University, but moved to Chicago in 1929, to remain there for the rest of his academic career, becoming an Emeritus Professor in 1962.

His major research was on the taxonomy of termites, regarded by the uninitiated merely as pests, but to Dr Emerson fascinating social creatures, posessing, as they do, a large measure of self-sacrifice, a trade union system that runs with a minimum of demarcation disputes, and very sophisticated engineering—some termites air-condition their nests by humidity and heat control (sometimes by using the metabolic heat producton of fungi deliberately brought into the nest for the purpose) and by the cunning design of interconnecting air shafts.

In his research he travelled extensively, living on occasion in tribal villages in the remote jungles of Borneo and the Congo, capturing, cataloguing and classifying termites. His collection, built up over half a century of expeditions, comprises of 1,645 species (to Emerson's chagrin, only 93 per cent. of the known world total) and is preserved, pickled in alcohol, in the Museum of Natural History in New York, to whom Emerson presented it in 1964. It has already proved invaluable to termitologists from all over the world. The fascination of the termite world is conveyed by a book *Termite City* which, together with his wife, née Eleanor Fish, he wrote for children.

This was just one of his numerous publications, dealing with his primary research, evolution (he was a proponent of the controversial but now largely discredited theory of the Superorganism) and ecology. He became interested in ecology long before it was fashionable; he was elected president of the Ecological Society of America in 1941, and, amongst the many honours showered on him, was named 'Eminent Ecologist' by them in 1967.

nature

January 20, 1977

Systems for sale; way of life comes free

For as long as nations have been going to war with each other, capturing the market of another country has been regarded as a desirable alternative to the bloody business of capturing its virgins, chests of gold, galleons or surface-to-air missiles. And whilst a favourable trade balance made up from a portfolio of exports ranging from pins to double-decker buses is a fairly good way of making a possible enemy into an ally out of necessity, a much better way, most governments seem to agree, is by exporting whole systems as opposed to single items.

The virtue of your system in someone else's territory is pretty obvious. First it needs installing: not really a job that the locals can do, so hordes of technicians are temporarily exported to roam unchallenged in the course of their technical duties. Then the locals have to be taught how to keep it going: schools are established and, with luck, some of your own way of life and thinking rubs off, along with all the know-how. Then there will be a continuing demand for spares—available, of course, only from the original manufacturers if the design is made exotic enough. There will be a steady stream of advisers, consultants, conferences and working parties. And when the system wears out, guess who has just the right second-generation system, tailor-made to requirements and ready to start the cycle all over again. It is clearly much more than just foreign exchange which such an arrangement brings in—it provides cultural links, a terrestrial toehold and, of course, political benefits.

In the past these super-exports have included legal systems, constitutions, drinking habits, land drainage, agricultural practices, railways, literacy schemes, communications networks, family planning programmes, meteorological services, health care, airlines and, inevitably, military systems of all shapes and sizes. Nor is there any sign that the pace in systems-exporting will slacken: indeed the newly-rich oil countries are a ready-made market, as every industrialised country knows. But what, besides the staple military diet, is likely to be an attractive system to sell in the 1980s and 1990s? One completely new item is likely to be the earthquake early-warning system.

Few can now be ignorant of the remarkable strides taken in earthquake prediction in the past ten years. Very few quakes have yet been accurately predicted, but the pace of scientific advance and the lessons now being learnt about public response in various parts of the world to the issuance of warnings make it almost inevitable that over the next few years a few countries, notably China, the Soviet Union, Japan and the United States are going to make major strides in learning how to save lives. But many of the most predictable earthquakes probably occur in other parts of the world—Turkey, Iran, the Philippines, Mexico, Central America, Peru, Chile and so on, and many of these countries look very attractive propositions to the politically-oriented travelling salesman.

A national earthquake warning system will require hundreds of widespread installations, the development of a data network and a lot of involvement of the local inhabitants, especially if the more unusual predictions of earthquakes, such as animal behaviour, assume importance. It will be seen to be a worthy altruistic enterprise, proof that the government is concerned. It will also cost a lot of money. It looks absolutely made-to-measure as an exportable system. The question is not so much whether but how.

If the 's' in its name means anything at all, there is surely a strong case for Unesco to take a long look at the problem. For although warning systems would undoubtedly be good things for many countries, these same countries can do without latter-day economic imperialism, often in the guise of overseas aid. An alternative means of delivering effective systems without the imperialist content will not be easy to find, but it is at least worth making a serious effort to do so at this stage. For time is getting short. □

Overdue: another scientific revolution

J. Tuzo Wilson, Director General of the Ontario Science Centre in Toronto, argues the case for a return from growth to stability

CERTAIN major turning points in the history of science can be called scientific revolutions. Their influence is not confined to science, for they have solved philosophical puzzles and changed economies, but their importance warrants a search for new revolutions. It might be fruitful: history shows that earlier scientific revolutions have cast their shadows before them and have progressed through several parallel stages.

Before each of the past revolutions, for example, their old theories worked well and were useful. Their value has been partly hidden by subsequent attempts to denigrate them: before Copernicus, Ptolemaic astronomers had invented the calendar, predicted eclipses and acted as navigators for Columbus and Magellan; biology, classical physics and geology were similarly useful and well-developed sciences, even before the revolutionary discoveries of Darwin, Becquerel and Wegener. The triumph of each of the new theories was due to important new discoveries, observations or ideas which the old theories could not explain, as the observation of the phases of Venus, the discovery of radioactivity and realisation of the significance of mid-ocean ridges and paleomagnetism all demonstrate.

In each case the great event marking the revolution revealed contradictions and introduced problems which the old theory could not resolve, leading to a period of confusion. For the most part it was not experts but rebels, outsiders and interlopers from other fields of science who suggested the need for a revolution and produced the evidence supporting it. Thus most geologists continued to oppose the concept of continental drift long after Wegener (a meteorologist) had championed it, after physicists had provided key evidence supporting it and after many of the public had accepted it.

So, far from welcoming the new ideas, the establishment, who had the most to lose, clung for as long as possible to the old, justifying their position by questioning the new data, discrediting those who advanced them, and trying to patch up the old theories. In these endeavours they frequently found themselves supporting quite illogical positions. Before each revolution was accepted, the state of the subject had become chaotic, but each solved many problems so that the ensuing periods were times of great scientific progress and material benefit.

When one now looks for a field ripe for a scientific revolution the only likely candidate appears to be the marginal science of economic theory. It seems to meet most of the criteria advanced. To the extent that the steady increase in wealth over the past few centuries can be attributed to theory, economic theory can be held to have been successful. But any examination of nature or of primitive societies or any consideration of the mathematical consequences of continuous growth will show that stability is normal and growth abnormal. The history of the West since the Renaissance, and particularly since the start of the Industrial Revolution, has been one of growth. It has made most of us forget that this is unusual and must end.

Now an event has occurred which is so important that it is altering the course which history has maintained for several centuries. The cause of the period of growth and the reason for a delay in fulfilling Malthus' predictions has been that for several centuries energy has become ever more abundant at ever less cost. The oil embargo and the quadrupling of the price of crude oil has ended this period and marks a turning point in history. Some say we must seek substitutes. This is not as important as many seem to think, because many are already available. What is needed is the recognition that no substitutes, either known or awaiting discovery, are at all likely to be so cheap. It seems probable that holes drilled in the ground to produce petroleum have been cheaper than any mines, factories or collecting devices which can be built to generate other forms of power are likely to be.

But matters go further. The old system has broken down. There is a recession from which economies are not emerging as the old cyclical pattern predicted. There is unemployment coupled with inflation. In addition, many organisations and individual members of the general public since Malthus have recognised the coming breakdown, seen that growth can only be temporary, and predicted change. It is not right to say that history has proved Malthus wrong. Fundamentally his ideas were correct, but living in the eighteenth century he did not see that development of power from steam, electricity and internal combustion engines and the whole industrial revolution would delay their impact.

Moreover, the establishment, blind to logic, denigrates those who foresee the inevitability of change as "doomsters", and clings to theories which if they have not broken down completely already are mathematically certain to do so before long. The differences between those who advocate growth and those who predict no-growth is not as great as might be imagined. The supposed economic experts all demand a return as quickly as possible to an annual rate of growth of, say, 5%. Politicians, businessmen and labour leaders all say that this can be attained, and they well may be right for the next year or two, which is as far as they look ahead, to the next election, next meeting of shareholders, or next signing of a union contract.

On the other hand, any accountant can calculate that an increase in demand of 5% annually will in thirty years produce more than a fourfold increase in demand. Thirty years is a period most people can expect to see, and one for which young people make plans, but a fourfold increase in demand is ridiculous in face of present shortages. Thus the position held by the present ruling establishment, as has happened in past revolutions, has become untenable. As an example, I was astonished that an influential man to whom I advanced these views replied that they could not be right because if true they would ruin business!

It will be difficult to convince businessmen, who for several centuries have flourished upon growth, that growth cannot long continue. It will be particularly distressing to them because in the past the declining cost of energy has rewarded innovation with profit, so that greed overcame man's natural lassitude and aversion to change. Each change—from slaves to animal power, from windpower to steam and from steam to internal combustion engines—increased profits.

In future the changes to more expensive forms of power will decrease profits and this is producing a tendency to cling to existing practices. If supplies become exhausted before this is overcome and adequate replacements made, breakdown and anarchy are likely to result. Therefore it is vital to prepare for a return to a stable society and not fritter away resources which are still available to us trying to stimulate growth when the means to support growth are no longer going to be available.

For each past revolution, however, recognition of the new theory has always made progress possible again. Even after it has been recognised that we must accept an overall economy which is less lavish than before, great rebuilding will be needed. As the supplies of oil and gas run out the whole petroleum industry, for example, will have to be replaced by other industries, ultimately by ones that depend upon renewable resources. We have seen many such changes, for example the change over the past 50 years from coal to oil. These changes involve work and employment.

No growth overall need not mean overall stagnation once the correct direction is recognised and followed. Undisturbed Nature is stable, but it is certainly active. I therefore prefer the term renewal economy to no-growth society. The best chance to avoid ultimate breakdown and anarchy and to maintain any of the present forms of society is to take steps at once to change them. It should be noted that the change in philosophy must be farther reaching than a mere change between one or another existing political party or between a planned society and free enterprise. All the present economic philosophies ignore Nature's and mathematics' edict that growth cannot continue indefinitely. If some extreme governments have achieved conditions of no growth in their countries it is because of their ineptitude, not their desire.

The need is imperative to recognise and to start to implement this great revolutionary return from growth to stability. Its effects are likely to be more traumatic than those of any previous scientific revolution, but can best be ameliorated by early recognition and acceptance. Nearly three years have already passed since the revolutionary turning point in the availability of energy occurred. It is ironic that it would appear that it is the conservative blindness of those who should be leaders, whether in politics, business, or labour, that is the greatest impediment to advance. □

Space: a new phase?

Keith Runcorn and **Paul Coleman** describe plans for a major new effort in exploration and urge more European cooperation

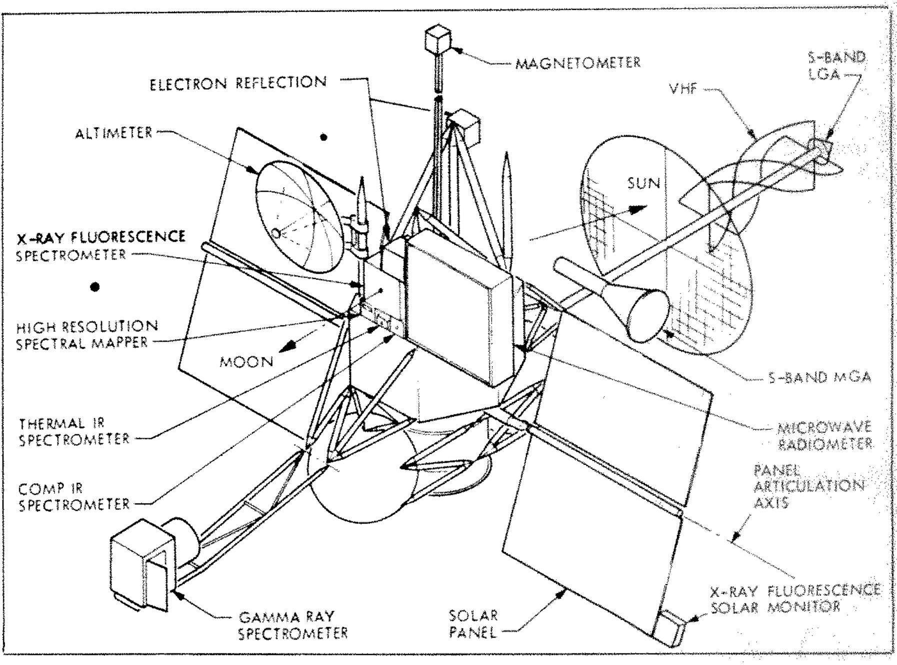
THE landings of Viking spacecraft on Mars on July 20 and September 4, 1976, and of Venera 9 and 10 on the surface of Venus in 1975, mark the culmination of a period of lunar and planetary exploration. Over the past decade manned Apollo landings and the unmanned Ranger, Surveyor, and Luna 16, 20 and 24 landings have been made on selected sites on the Moon. From the data collected on all these remarkable missions, our understanding of the evolution and present state of the Moon and planets is developing at an unprecedented rate. The next decade will see a revolution in our knowledge of the solar system comparable with those studies of continental drift and plate tectonics which, over the past two decades, have transformed our understanding of the Earth.

Europe, with a combined gross national product greater than that of the USA and the Soviet Union, has not so far taken a role which reflects its strength in this field, one of mankind's greatest explorations and scientific endeavours. An opportunity will be afforded by the International Solar System Programme, a project of international collaboration in the exploration of the solar system. The International Council of Scientific Unions recommended last year that all countries should consider how they can most effectively contribute.

Our knowledge of the Moon exceeds in its detail that of any other body in the solar system except the Earth. While it might be feared that sampling the Moon in a restricted number of sites might hopelessly bias our interpretations, it is now clear that the absence of an atmosphere and an ocean and the weaker tectonic forces than on Earth have resulted in much less segregation and concentration of elements and a much smaller variety of rock strata than on the Earth. This has in turn made it possible for the relatively small number of sampling sites to yield data reasonably representative of the Moon as a whole. On terrestrial planets limited samples might not be as successful in determining global properties.

The Moon differentiated about 4.4×10^9 years ago, forming an anorthositic gabbroic crust apparently thicker on the far side than the near side. The sources of the mare basalts, (crystallisation ages 3.2 to 3.8×10^9 years) which filled the great impact basins left in the Moon after catastrophic bombardment, seem to have differentiated at the same time. The processes that brought about this differentiation are obscure and the depth to which it extended is unknown. If the Moon is found to possess an iron core, this early differentiation must have occurred throughout the Moon. The siderophile and volatile elements on the Moon are depleted relative to the Earth and meteorites: information potentially a key to our understanding of the early evolution and origin of the Moon.

Three entirely unexpected charac-



JPL

Flight configuration for TBOL

Keith Runcorn is at the University of Newcastle-upon-Tyne. Paul Coleman is at the University of California, Los Angeles.

teristics were established from physical studies: large positive gravity anomalies over the circular mare (the mascons), moonquakes both deep and shallow, and widespread magnetisation of the crust. The interpretations of these discoveries are still controversial but we conclude that in its early history the Moon was much more active internally than it is today. If so, why has the Moon died?

The interpretation of gravity field on the near side requires an equally complete survey on the far side. Basins of comparable size to the mare are known to exist on the far side, but they are not filled with lava and whether they have negative or positive gravity anomalies is uncertain. Thus, the exact delineation of gravity above these far-side basins is of critical importance to the understanding of the process which resulted in the flooding of mare and the production of mascons on the near side.

Again, the controversy concerning the interpretation of the magnetism of lunar rock centres upon whether the moon generated, between 4×10^9 years and 3.2×10^9 years ago, a general magnetic field which it does not have today. The direction of magnetisation of the lunar crust was impossible to determine by the methods used in terrestrial paleomagnetism; the Apollo samples were unoriented. Instead, interpretation of magnetic anomalies must be used. The existence of an iron core in which a dynamo process might once have been active remains in doubt. A more accurate value of the moment of inertia factor (0.4 for a uniform sphere) which is now given as .392±.003 could resolve this particular matter.

The definitive solutions of many interesting problems raised by existing data depend on completing the survey of the moon which has been so well begun. To this end the lunar polar orbiter has been conceived in a mission named at present the Terrestrial Bodies Orbiter–Lunar (TBOL). A satellite carrying a variety of remote sensors would be put in lunar orbit about 100 kilometres above the surface so that a complete and relatively detailed sur-

The experimental programme

The geochemical experiments consist of a solid state (germanium) counter for gamma rays and four proportional counters (three looking at the moon and one at the sun) for X rays. The former will detect both the radioactive nuclides K, Th, U and the elements Fe, O, Si, Ti, Mg, Al, Ca (in declining order of detectability) excited by the galactic cosmic-ray neutrons and by solar flares. The latter will detect the characteristic X rays emitted from Al, Si, Mg for the quiescent sun and also Ca, K, Fe, Ti during flares. The sampling depth will be 15 cm for the gamma rays and 10 μm for the X rays and spatial resolutions will be 50 km and 10 km, respectively. These experiments will provide much more detailed elemental abundance data than has so far been available and will contribute to the understanding of the differentiation of the moon, especially of the highland regions thought to be anorthositic gabbroic in character but only on the basis of fragmentary samples.

Water of the moon, perhaps retained in the regolith of deep craters near the poles, is exciting much interest and the gamma ray experiment may detect it from the 2.22 MeV line de-excitation of deuterium produced by slow neutrons created in material on the lunar surface by cosmic-ray bombardment.

The radioactivity is known to be greater in the western mare, so that an experiment to detect the surface heat flow is proposed. It may be surprising that this can be done from orbit, but microwave radiometers (3 cm, 10 cm, 20 cm)—three identical beams with 50 km resolution—detect the black body radiation from different depths (down to about 5 m) in the regolith where, because of its extremely low conductivity, the vertical temperature gradient is large. These radiometers and infrared detectors will also measure the lunar daily inflow and outflow of solar radiation, from which the thermal conductivity of the regolith can be calculated. Thus the distribution of heat sources within the moon will be globally mapped and hypotheses such as convection in the interior will be tested.

Reflectance spectroscopy techniques over wavelengths 0.35–2.5 μm will be used to determine the mineralogical composition. This method has already been used effectively to determine the composition of the major satellites of planets. The wavelength of absorption lines from the excitation of electrons from Fe^{++}, Fe^{+++} and ions of other transition metals are influenced by the crystalline structure of the SiO lattice. It will be supplemented by spectro-stereo images with six filters. The latter instrument, of course, will also provide, in high resolution (~100 m), further details of the morphology, stratigraphy and, through crater counts, chronology of the lunar surface.

Magnetic maps of the moon will be obtained at the orbital altitude by a 3-component magnetometer accurate to 0.1 γ and at the surface by an electrostatic analyser of electron fluxes. The latter will detect low energy electrons, spiralling around the lines of the solar wind magnetic field, which are reflected from the convergence of the field lines produced by surface magnetic anomalies. These two methods will complement each other as crustal magnetic anomalies having sharp contrasts of magnetisation look very different at the surface and at heights of 100 km. As various interesting explanations of the magnetisation of lunar rocks have been suggested, some involving cometary and meteoritic impacts, data on the general morphology of magnetic anomalies may establish whether or not these external sources or a magnetic field originating at the lunar core are responsible.

The existence of a lunar core can also be investigated by observing the electromagnetic response of the moon as it enters the fairly steady geomagnetic tail field. The core acts initially as diamagnetic and then the induced dipole moment begins to decay—from this initial decay the free decay time constant of the electric currents in the core can be determined.

The map of the gravity field on the near side will be improved by tracking of the orbiting satellite. For the far side of the moon, complete maps will be made of the gravity field, of which at present nothing is known except by inference from the evolution of the orbital elements of the subsatellites launched in the Apollo 15 and 16 missions. The closely related study of the figure of the moon has been controversial, as the coverage of the laser altimeters on the Apollo 15, 16 and 17 missions has been very limited and the classical earth-bound method, using the geometrical librations which enable height determinations to be made by the parallax principle, were extremely noisy. The radar altimeter in the orbiter should enable the surface to be mapped completely to a high accuracy. The moon departs from a sphere in a variety of ways and the mapping of the moon will be very important to refine our understanding of its non-hydrostatic shape.

vey would be possible. A radio-relay satellite would be put into a more distant orbit to provide an almost continuous radio contact between the instrumented polar orbiter and the Earth. Such a mission, in which the scientific aspects have already been developed (see figure and box), is under consideration by NASA for a 1982 launch (see following story).

The Terrestrial Bodies Orbiter–Lunar mission is part of a new phase of planetary exploration, by means of remote-sensing orbiting spacecraft. A somewhat similar approach, but with a spinning spacecraft, is now under way in the United States for exploration of Venus, and a Jupiter orbiter is in the planning stages. These latter two missions will also involve atmospheric probes, which are unnecessary for planets and bodies without a substantial atmosphere. Modified versions of the TBOL spacecraft could extend further the exploration of such bodies in future.

More active European participation at this crucial stage in the exploration of the solar system would be timely and would enable European scientists, who contributed so much to the analysis of lunar samples, to revive the old scientific tradition of which we have been reminded in recent years by the anniversaries of Copernicus, Kepler and Galileo. □

USA

Ford's parting gifts

Colin Norman in Washington reports on the proposals for research and development in Mr Ford's third and final budget

WITH just three days of his brief and troubled Presidency left to run, Mr Ford this week presented Congress with a complex and detailed set of budget proposals for the 1978 Fiscal Year, which begins on October 1, 1977, more than eight months after he leaves office. Though they are likely to be substantially revised, both by the incoming Carter Administration and by the Democratic-controlled Congress, Mr Ford's parting economic proposals could result in substantial increases in support for science and technology, particularly for basic research.

The proposals represent the lame-duck Administration's detailed prescriptions for limiting growth in Federal spending, reforming the sprawling government bureaucracy and setting priorities among Federal programmes. During the next few weeks, President Carter and his economic advisers will sift through Mr Ford's budget and offer a raft of amendments to bring it into line with the new administration's economic and political philosophies. Carter will concentrate on the more political proposals, however, and it is likely that many of the less controversial programmes, which include most concerned with science and technology, will survive the change of administration more or less intact.

Mr Ford's budget includes a total of about $26,300 million for research and development, an increase of about 8% over estimated expenditures this year. As in the past few years, defence and energy research and development account for the bulk of the increases, but basic research is also singled out for favourable treatment, rising by about 9%, to reach some $3,000 million. Inflation is expected to hover around 6%, so the Ford budget proposals should result in a modest, but real, growth in Federal research support.

According to a discussion paper published by the Office of Management and Budget, funding for basic research in the United States has declined by about 27% over the past decade, chiefly because funding increases have not kept pace with inflation. Although the document suggests that basic science in the United States is competitive with that of other countries, the decline in support "has raised concerns about whether the United States might not be underinvesting in basic research to the detriment of the nation's future".

The chief beneficiary of the proposed increases is the National Science Foundation (NSF), scheduled for an increase in funds for some 12%. According to Richard Atkinson, the acting director of NSF, a major use of the proposed increase will be to upgrade scientific instruments and equipment in universities and colleges. NSF's total budget is set to climb to $885 million under Mr Ford's proposals.

The budget is the first to be produced since the establishment of the White House Office of Science and Technology Policy (OSTP), and some credit for the proposed increases is being claimed for the new arrangement. H. Guyford Stever, the head of OSTP and Mr Ford's science adviser (who will also leave the Federal Government this week) notes with some satisfaction that "although it is a constrained budget, it is not as constrained as in previous years". Asked whether he expects the Carter Administration to go along with the proposals, Stever pointed out that few of the research and development proposals have political implications, and he suggested that "if the incoming administration asks for advice on the research and development budget it would get much the same advice" as Mr Ford received. Since Mr Carter had not chosen a replacement for Dr Stever at the beginning of this week, he does not seem to feel an urgent need for such advice.

Included in the budget proposals are a number of new programmes which have been waiting in the wings for some time, and which are likely to meet with approval from the Carter Administration. Prominent among them is a major effort in earthquake research. A three-year project divided between the National Science Foundation and the Department of the Interior, it is expected to receive about $150 million, with the first instalment of $23 million coming this year. The goals are "development of a reliable prediction capability within 10 years through increased research on fundamental causes of earthquakes and precursor phenomena", establishment of building codes for structures in earthquake prone areas, publication of regional hazard assessment maps, and studies of the social, legal and economic implications of reliable earthquake prediction capability. Such a programme was recommended last year by a White House Advisory Committee, and a similar effort was incorporated in a bill passed last year by the Senate. The Carter Administration is unlikely to reject the proposal.

Another area to receive some long-overdue support in Mr Ford's budget is agricultural research. Some $28 million has been proposed for a new programme of basic research on photosynthesis, nitrogen fixation, crop productivity, and genetic engineering of crop plants, funded through a system of competitive grants similar to those operated by the National Science Foundation and the National Institutes of Health. At present, virtually all the research supported by the Department of Agriculture is funded through block grants to institutions, and initiation of a competing grants programme has long been recommended, particularly by committees of the National Academy of Sciences, as a way to increase the quality of agricultural research. The proposed programme is virtually certain to receive a warm reception in Congress since the House last year approved a bill designed to initiate just such a programme.

Table 5 Conduct of basic research by major departments and agencies (in millions of dollars)

Department or agency	Obligations 1976 actual	Obligations 1977 estimate	Obligations 1978 estimate	Outlays 1976 actual	Outlays 1977 estimate	Outlays 1978 estimate
Health, Education and Welfare	660	744	796	694	644	745
(National Institutes of Health)	(590)	(659)	(710)	(627)	(568)	(662)
National Science Foundation	541	612	688	534	574	650
Energy Research and Development Administration	346	389	427	330	370	413
National Aeronautics and Space Administration	298	352	365	297	321	360
Defence—Military functions	248	274	314	225	279	314
Agriculture	171	193	215	168	200	210
Interior	121	127	143	125	125	137
Smithsonian	26	31	32	28	31	31
Commerce	22	25	24	21	24	25
Environmental Protection Agency	14	21	18	13	21	14
All other	16	17	19	16	15	20
Total	**2,463**	**2,785**	**3,041**	**2,451**	**2,604**	**2,919**

Space science is also singled out for a modest increase in Mr Ford's proposed budget. A new start has been proposed on the construction of the Large Space Telescope (LST), a 2.4-metre optical telescope which is expected to be launched by the space shuttle in 1983. Estimated to cost a total of between 435 and 470 million dollars, the LST has consistently been ranked as a top priority space science project by the National Academy of Sciences, but restrictions on federal expenditures in the past two years have twice caused the programme to be deferred. Funds have also been proposed for another high-priority mission, construction of a spacecraft to orbit Jupiter and send probes into the planet's atmosphere. And finally, Mr Ford's budget includes funds to begin construction of three more shuttle orbiters, in addition to the two which have already been approved. There are, however, some casualties in NASA's budget, most prominent of which is a proposal to place a spacecraft in a polar orbit around the moon (see page 197). A start on that mission was anticipated in the 1978 Fiscal Year, but it has been deferred, at least until 1979. So far, Mr Carter has made few public comments about the space programme; thus the fate of Mr Ford's proposals for NASA are uncertain. The projected total costs of the LST may, however, make it a plum target and there is some speculation that Carter will ask NASA to seek foreign financial participation in the project.

One area of Mr Ford's research and development budget which is likely to be substantially modified by the Carter Administration is the proposed funding for energy research and development. Though Mr. Ford has recommended a hefty increase in total support for energy programmes, with some $3,000 million included in the budget for research and development, much of the additional money would be swallowed up by nuclear energy research efforts. The Liquid Metal Fast Breeder Reactor (LMFBR) programme alone is scheduled to increase from 686 to 855 million dollars. During the election campaign, Carter promised to reduce the priority of the LMFBR project and to seek international participation in it. He also consistently promised to try to reduce United States' reliance on nuclear power.

A particular focus of conflict between Ford's energy proposals and Carter's election promises is likely to involve programmes designed to increase energy conservation and to spur commercial introduction of solar heating devices. Mr Ford's budget would involve virtually no increase in funds for research and development on energy conservation, chiefly because the Ford Administration believes such efforts should be the responsibility of private industry rather than the federal government. Similarly, the proposed Ford budget would reduce federal support for a programme designed to provide subsidies for the production and installation of solar heating devices. Again, the argument is that the technology is relatively well developed and private industry should bear the chief responsibility for its commercialisation. Throughout the election campaign, Mr Carter frequently promised to make energy conservation and the development of alternative sources of energy his top priorities. Moreover, James Schlesinger, the former head of the Atomic Energy Commission, whom Carter has named as his chief energy adviser, has also argued strongly for increased efforts to stimulate energy conservation. The thorny question of government involvement in private industry, and Federal subsidies to spur commercial development of those technologies, will clearly have to be addressed very soon by the new president and his energy team. As for fusion energy, Mr Ford's budget provides funds for construction of a large Tokamak device at Princeton University—a project which was reported to be in jeopardy at one stage in the budget deliberations—and it also contains funds to increase the already sizeable fusion project at Lawrence Livermore Laboratory.

Another area which is likely to be a source of controversy is support for biomedical research. The Ford budget provides very little increase in support for biomedical research funded by the National Institutes of Health (NIH), and in particular it seeks to hold the budgets of the National Cancer Institute and the National Heart and Lung Institute approximately constant, after several years of rapid growth. There is also a proposal to transfer responsibility for NIH's intramural research to the Assistant Secretary for Health, a suggestion which is likely to raise considerable protest from biomedical researchers since it smacks of increased political control over health research.

Ford tried to hold down spending by NIH in his two previous budgets but Congress has refused to go along with the reductions and instead it has voted hefty increases for biomedical research. The same pattern is likely to be repeated this year, but the Carter Administration will soon be forced to take a close look at priorities in biomedical research. In that regard, a study produced last year by a presidential commission recommended that there should be some changes in the privileged political position of the National Cancer Institute, but the recommendations have so far been neglected.

Finally, it is worth noting that when President Ford entered the White House in August 1974, he inherited, among other troubles, a scientific community which had been complaining bitterly about declining research support and the lack of a central science policy office. In his brief term of office, Ford has taken several steps to attend to the concerns of the scientific community, and when he leaves the White House this week, he will bequeath his successor a much more healthy scientific legacy. ☐

SWEDEN

Reforming the Councils

Wendy Barnaby reports from Stockholm on proposals to reform Sweden's Research Councils

THE reform of Sweden's Research Councils due to go into effect on July 1 this year is basically an attempt to set up a structure which will accommodate both society's need for research that produces usable and useful results, and the scientists' traditional demand for freedom in conducting research. Of course, any attempt to fuse these two is only as good as the mechanisms whereby fusion is to be brought about—and the will of the people involved to make it stick. But the mechanisms, at least as explained by Mr Jan Stiernstedt, Permanent Secretary of the Ministry of Education and chairman of the reform's organisational committee, look like a model example of the Swedish genius for balance and compromise.

Within the sphere of the Ministry of Education there are at the moment five Research Councils (Humanistic, Social Science, Medical, Atomic and Natural Science), with an average membership of eighteen plus a chairman. In the budget year 1975–76 they were allocated about 6% of the state's expenditure on research and development, which itself made up 3.3% of the total budget, so they are hardly financial giants. In fact, as far as research and development goes, they embody precisely that weighty meagreness which is the essence of prestige—and that they certainly have. Their various structures have resulted from haphazard growth reflecting the enormous post-war increase in the breadth and complexity of research; the reform aims to tidy them up organisationally as well as to make them more sensitive to public interests.

It envisages only three councils (Humanistic and Social Science, Medical and Natural Science), each with ten members who will hold office for six years, and a chairman. The actual number of members will thus have been reduced to a third of its present total, an attempt to make the new bodies more effective. At the same time, however, they are to be made as representative as possible of the views of the academics in each field. Seven members of each council are to be elected from the universities and institutes of higher education, where small assemblies will be elected by those members of staff who have a

USSR

● The recent announcement that the Soviet Union will sell 200 tonnes of heavy water to India for use in the Rajasthan Atomic Power Plant reveals a certain modification in Soviet policy, regarding both aid to India and the non-proliferation of potential nuclear armaments.

Although the first Soviet–Indian mutual cooperation agreement is now more than five years old, India has until now received relatively little technological aid from the Soviet Union, either as hardware or expertise. During the visit of Mrs Indira Gandhi to Moscow in June, 1976, the 'strengthening' of such cooperation was one of the main topics of discussion. The heavy water sale would appear to be one of the fruits of these talks.

Heavy water is, of course, a sensitive topic as far as India is concerned. Although she can produce a small amount of her own, this is not sufficient to supply an installation the size of the Rajasthan power station. Originally, India obtained heavy water from the USA and Canada; the connection with India's explosion of a nuclear device led Canada to cut off supplies, and later to make the ban permanent following unsuccessful negotiations on inspection and safeguards.

The Soviet Union has always shown considerable caution regarding nuclear exports. A recent *Pravda* article, while not referring directly to the Indian deal, emphasises the role of the Soviet Union, within the IAEA, the "London Club" of nuclear suppliers and the United Nations, in "organising monitoring operations". The article stresses the need to increase the efficiency of such international control, and quotes the Soviet non-proliferation memorandum to the 31st UN General Assembly emphasising the "special responsibility" of the nuclear powers and the need for "strict guarantees that international cooperation in the peaceful use of nuclear energy should not become a channel for the proliferation of nuclear armaments".

The Soviet Union has presumably found some way to extract from India the kind of guarantees which, after two years' negotiation, Canada was unable to obtain.

● The filling of the Nurek reservoir on the river Vakhsh in Tadzhikstan has been halted at the 140 m mark on the 300 m dam, on the advice of a joint Soviet–US geophysical team now working in Tadzhikstan under the terms of the Nixon–Brezhnev cooperation agreement.

Commenting on the decision, the Academic Secretary of the Soviet Academy of Sciences, I. Ivanov, noted that the dam is situated in the only region of the continental part of the globe where as many as 2,000 tremors occur annually. Filling of the dam has produced a marked increase in seismicity and, according, further raising of the water-level will take place very gradually. There is no suggestion, however, that the planned final capacity of the reservoir will be reduced.

● One of the anomalies of Dr Veniamin Levich's position as a *refusnik* is that although he is no longer permitted to work as a scientist he still retains his position as a Corresponding Member of the Academy of Sciences. The Academy itself still retains a certain autonomy, and according to its statutes requires a two-thirds majority vote in secret ballot for the expulsion of a member. When an Academy meeting to discuss draft changes in its rules was called for December 22, 1976, Academician Levich wrote to say that he wished to speak at the meeting, to propose a statement clarifying the moral responsibility of Academy members. He was informed in due course that the said meeting had been postponed until February 1977, "when this proposal will be entered".

In proposing the statement, Levich clearly wished to draw attention to the plight of dissident scientists. It should be noted, however, that the last time a number of Soviet Academicians made a declaration on 'moral responsibility'—as individuals, and not as a corporate body—was during the 1973 press campaign against Andrei Sakharov. They declared then that the first duty of a Soviet scientist was uncompromising support for the State.

Vera Rich

PhD. These local bodies will together form a national assembly, which will then elect the council members. The other three members will be appointed by the government and will represent the various official bodies that exist here within each field: the Environment Protection Board and the Board of Education, for example, within the humanities and social sciences. The chairman will be appointed by the government. Each council is thus to embody the principle of scientific freedom by having members drawn only from within the field. The fact that a majority of members will be elected by active researchers will bend the councils in the direction of current interests.

But what happens if enthusiasm for current research interests solidifies into unwillingness to risk failure with newer or simply unfashionable ideas? This is a criticism made by one prominent Swedish medical scientist of the existing Medical Research Council. It is precisely to prevent the conventional wisdom of the dominating group within any field from restricting other impulses that the reform was undertaken. And as the impulses to be encouraged are those which come from the broader society, the main innovation of the reform is the creation of a special body to give them a persuasive voice—the Research Councils Coordinating Board.

This will exist alongside the councils and have two main tasks: to coordinate administrative procedures amongst them and to promote cross-disciplinary research projects of interest to society in general and needing cooperation between the different councils, institutes and boards. It will consist of a chairman, to be appointed by the government, and twelve other members, one to be appointed from each of the three councils, the Agricultural and Forestry Research Council and the Board for Technical Development, and the other seven to be appointed by the government as elected representatives of the community. At least four of them are to be members of parliament, and the others will come from local government and trade unions. In order to back up its suggestions, the Board will dispose of funds estimated to total at least Sk30 million (nearly US$57 million) by the budget year 1979–80.

Attempts to involve parliamentarians in Swedish science policy have so far been remarkably dull. But they have never been underwritten with funds, and money, as Mr Stiernstedt points out, is to be the key to the new Board's influence. If the Board can offer to fund a project, organisational inertia will in many cases be overcome. The Board will be able to stimulate but not dictate; every council will retain the right to decide which projects it will participate in. Finally, then, the scientists—if they want to—will be able to discount the Board's suggestions. On the other hand, they could make use of its resources to widen not only society's outlook, but also their own. ☐

NETHERLANDS

Satellite plans

Casper Schuuring reports from Holland on the latest Dutch commitment to space research and astronomy

AT the end of 1976 the Dutch government finally decided to finance a second astronomical satellite, to be launched by NASA with a Thor Delta rocket in the spring of 1981 from the Western Test Range in California. After the success of ANS, the first Astronomical Netherlands Satellite, in the field of soft and hard X-ray and ultraviolet radiation, the second satellite (IRAS) will do observations in the infrared.

The project started in January 1975 with a preliminary definition study. When contacts were sought for the scientific programme with NASA and the European Space Agency (ESA), there was an enthusiastic response, especially from the US community, at the prospect of stimulating this branch of astronomy. Following a NASA "announcement of opportunity" 13 proposals for experiments came in. A US study team consisting of 11 astronomers started discussions on the IRAS mission with their Dutch colleagues.

At the beginning of 1976 agreement was reached on the scientific programme and NASA expected a memorandum of understanding between the US and the Netherlands to be signed before July, 1976. The Dutch government agreed in principle to the project in July, in spite of a curtailment of expenditure generally, and it took six months to find the necessary money.

The United States and the Netherlands will both have to pay around 110 million guilders (£1 = DG 4.15). Out of the discussions with the ESA, moreover, a British interest arose, and the UK Science Research Council took an active part in the US–Dutch negotiations. Britain is contributing 10 million guilders, for the most part consisting of facilities for flight operations in the Appleton Laboratories in Slough.

The design and building of the satellite represents a challenge to Dutch industry, and especially Philips and Fokker-VFW, which hope to continue the industrial innovation which resulted from the ANS project. A British advisory group, General Technology Systems Ltd, provided evaluation of the possible profits of space activities for the Netherlands in the coming years and of the IRAS project in particular. The results of this study are said to be favourable and support the new project.

The great amount of data from IRAS will make higher demands on storage and retrieval than with ANS. Large scale integration techniques will be used in the on-board computer, giving it a maximum of calculating capacity (500 million bits per day). Also of an advanced design is the extreme cooling system for the telescope and the detectors. Infrared observations are only possible if the instruments are cooled close to absolute zero. A special helium-cooled vessel has therefore been developed.

The results of the IRAS mission, the plan of which was set up in June, 1975 by a commission of 15 American, British and Dutch astronomers, will be received in time to be used for the detailed programming of the experiments with Spacelab in the 1980s. Over a six-month period a survey will be made of infrared sources in the Milky Way and infrared radiation from extra galactic systems. In the following six months more detailed measurements on different sources will be carried out. The astronomers hope to trace and map some 10 million infrared sources. The 925 kg satellite will fly in a circular polar trajectory: one earth orbit will take 124 minutes, so that a sun-synchronous trajectory on a height of 900 km is followed.

Not everybody is as enthusiastic as the astronomers, however. Dutch associations of scientific workers protested in 1974 against further Dutch space activities. At that time an agricultural satellite for underdeveloped countries was under study, as well as the IRAS. This project was not then as far advanced as IRAS, and the benefits for these countries from it were considered small because they lacked the necessary infrastructure. The associations were also against IRAS, as "this type of research would hardly contribute to solve the real problems in the world". ☐

IN BRIEF

Computing costs

For the second time in three weeks the UK Computer Board for Research Councils and Universities has drawn attention to the problem it is facing with rising costs. In its Sixth Annual Report published just before Christmas (Cmnd. 6696, HMSO, 45p), the Board expressed concern at the rapid increase in recurrent expenditure at university computing centres. These had resulted mainly from staff salary rises and increased maintenance costs. The Board added that it was "unlikely" that all recurrent commitments could be met, and that criteria were being sought for the equitable allocation of the funds available. This is expected to be a tortuous process.

Last week the Board published a report outlining the policy framework the Board considers necessary for the development of university computing facilities over the next ten years (*Computers in Higher Education and Research: The Next Decade*, HMSO, 60p). Concern about costs was again emphasised but the cause became clearer when a figure was put on the rise. From the 1975–76 level of £6.2 million, the trend is for recurrent costs to rise—"at a rate of 32%". And the figure is an annual one, though the report does not make this clear.

The Board estimates that university computing facilities proposed for the next decade could be obtained at a capital cost of £9.4 million a year. It adds that "it is not entirely clear" that the government's procurement policy favouring ICL has been beneficial "either to the universities or to ICL".

Uranium views

If opposition in Australia to the development of nuclear fuel resources is successful, scientists who want to become part of the development of uranium mining and processing and of nuclear power will have to leave the country, according to Sir Charles Court, the premier of Western Australia. Responding last week to scientists who have urged the national government to ban uranium mining and exports, he described their attitude as "negative and defeatist".

Aspects of the mining and processing of uranium were the subject of a symposium held in London this week. One presentation, from Michael Davis of the European Commission of the EEC, argued that European uranium needs in relation to the world market were of paramount importance to the market's future. "Close collaboration" between utilities and mining companies was highly desirable, and a contribution from the public authorities was necessary to achieve an orderly market.

Peer review

THOMAS H. JUKES

It has been said that the warp that holds the complex fabric of science together is peer review, and the woof is the noise made by scientists who complain about it. Be that as it may, it is obvious that without peer review, scientific literature would become a Tower of Babel. One has only to look across the street to see what goes on in the newspapers for reassurance as to this point. Science is essentially hierarchical; its progress and its integrity depend on the existence of an 'establishment', and on the rejection of uncontrolled or unrepeatable experimental results. There are objectors who say that such rigidity prevents valuable innovations from coming to light. The answer to this may be formulated as a Darwinian analogy: such innovations are like the exceedingly rare class of mutations that are beneficial to a species, and hence overcome all odds against their survival and spread.

I am indebted to a lecture by Professor Emilio Segre for an anecdote of Rutherford's rejection, on behalf of *Philosophical Magazine*, of a manuscript by the youthful Bohr, describing a new theory of atomic structure. But the young genius was not to be denied. He journeyed from Denmark to England to confront Rutherford, the giant of physics, and won his point: the famous article was published. The answer to the line in Gray's *Elegy*, mourning the possible loss of "some mute inglorious Milton", may be that Miltons are never mute. Nevertheless, I do not recommend making a trip to the offices of *Nature* to argue with the Editor except under most extreme circumstances.

The word "peer" implies acceptance by a group of equals. The process of acceptance in the group, and acknowledgement of its hegemony by the individual who has been accepted, is complex and fascinating. There are special cases; for example, one of the principles of scientific organisation is that a veterinarian should report only to another veterinarian. I presume that at the head of such a pyramid must be one, perhaps a Dr Cabot, D.V.M., who reports only to the Great Veterinarian. Nobel Laureates are another special case; some of them acquire omniscience after their return from Stockholm, and henceforth they blossom in new fields. This subject is admittedly a delicate one, but I am intrigued by an advertisement in a financial newspaper, appealing for tax-deductible contributions. "Each contributor will be sent an article on sugar—its effect on the body; an article on Vitamin C—how much to take and how it may benefit your life; and a just-published article showing the dramatic effects of Vitamin C on cancer." Idly, I wondered who reviewed these articles that are so temptingly offered in exchange for cash.

Publishing a book is a way of avoiding peer review. Confronted with criticism, the authors of such books are apt to remind their detractors that Galileo was persecuted by the Inquisition, and that sceptics told the Wright brothers that their aeroplane wouldn't fly. In one recent case, the author said that high-quality DNA and RNA can be supplied from outside the body to "nourish our cells and return them to a healthy state." Apparently he had not heard about nucleases, purine catabolism or gout. The publishers of the book, more interested in sales than veracity, allegedly have set aside $20,000 to promote it further. Peer review is evidently of secondary interest when such financial manoeuvres are involved.

And so, to those who chafe under the pettifogging and nitpicking annoyances of the comments of reviewers who "don't seem to understand how important this piece of research is", let us counsel patience, forbearance and cunning. Hang in there, and remember that, after all, if you can't convince the reviewer, you *may* not be able to persuade other readers. Also, you, too, may perhaps be a reviewer some day.

correspondence

Earthquake risk

SIR,—The succession of devastating earthquakes in recent months has vividly borne out O'Keefe *et al*'s conclusion[1] that "natural" disasters are on the increase. More remains to be learned about precursory phenomena before earthquakes can be forecast reliably enough to save lives regularly. Yet much can be done in other ways to mitigate the hazards. Earthquake risk is highest where the probability of earthquake occurrence and the vulnerability of an area combine to maximum effect, and in practical terms it is this that requires quantitative evaluation. Molchan *et al.*[2] have developed a mathematical analysis of earthquake risk but whereas evaluation of earthquake occurrence has attracted considerable attention, through compilation of seismic zoning maps and so on, the evaluation of vulnerability has received much less. Vulnerability is the distribution of loss potential, measured in financial terms, and is given by the actuarial assessment of the damage potential in an area both of material losses and social losses (loss of life, injury, loss of work, family, social disruption).

Planning must attempt to reduce vulnerability in areas of high seismicity, but it would be impracticable to design for total invulnerability. Hence, a measure of degree of vulnerability is needed. Engineers use the concept of earthquake resistance in structural design. I propose a similar concept of earthquake resilience should be used in the same way to assess the social and economic response. Just as engineers accept that some degree of damage to structures in an earthquake must be tolerated, so it must be accepted that some social losses have to be faced. Certain essential services such as hospitals, and fire fighting units may need special protection, both in terms of buildings and personnel. But other structures and services may perhaps be disrupted without long term detriment to the society. The concept of resilience provides a means of estimating the power of recovery of society in the event of an earthquake. With this methodology, the balance can be worked out for an acceptable expenditure to provide a tolerable degree of protection against an earthquake, which can be afforded within the economy of the country as a whole and which forms an integral part of its national development plan.

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[1] O'Keefe, P., Westgate, K. and Wisner, B. "Taking the naturalness out of natural disasters," *Nature*, **260**, 566–567 (1976).

[2] Molchan, G. M., Keilis-Borok, V. I., and Vilkovich, G. S., "Seismicity and principal seismic effects," *Geophys. J. R. astr. Soc.*, **21**, 323–335 (1970).

Significant journals of science

SIR,—The review article of Garfield (December 16, 1976, pages 609–615) contains relevant information in Table 3, where the impact factor (that is, the ratio between the citations in the 1974 Science Citation Index and articles published) of scientific journals is being presented in three specialities. Tables 1 and 2, on the other hand, are misleading. The ranking is by total number of citations. This criterion is invalid for a cross-section of all scientific journals.

In a field, say general medicine, where as a matter of example 10,000 workers each publish a paper with on average 10 references, this means 100,000 citations; material science, with 100 investigators and the same ratio, would yield only 1,000 citations. Thus the total number of citations, and in some lesser measure the "impact" too, are influenced by the total number of people interested in a given speciality.

Of the many factors influencing the number of laboratories working in any given field (besides fashion, inertia and others), last but not least, public funding must also be mentioned. The number of citations is rather a measure of the financial endowment of the field and not of the "significance" of the journal.

The 206 journals cover well established and thoroughly ploughed fields. Somewhat speciously it could be argued that for correlative, innovative, out-of-the-common and modern topics, which have not had time to accumulate citations and large teams, one must look outside of the 206.

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Rapid publication

SIR,—Rapid publication has become a topic of increasing importance as the camera-ready method and related methods have become more and more popular, especially for papers presented at symposia and the like. The manuscripts must be prepared by the authors according to special instructions. In small institutions the authors have to do all the processing themselves in addition to preparing the normal manuscript. They soon find out that a paper of 5–6 pages will take three to four weeks of drawing, photographing, editing, and typing according to the instructions. At a symposium with 200 to 250 papers, I would guess at least 100 will be prepared by the authors. This means that over 7 years are spent by scientists in preparing the camera-ready manuscript!

Institutions in developing countries usually tend to be small and have small budgets, and scientists working there are already overloaded with a wide variety of problems inherent in such situations. The preparation of camera-ready manuscripts therefore lessens the costly time which could be spent on actual research. Compared to scientists in well-equipped institutions, the scientists in small institutions see their research activities fall increasingly into arrears. I think that for us and other scientists in similar situations the advantages do not always equal the time and misery of preparing a camera-ready manuscript. I realise that the advantages for editors as well as for well-equipped scientists are quite substantial.

In case camera-ready manuscripts are required, I would suggest that possibilities for technical assistance are created in this respect for those who do not have it, and for which biologists are certainly not trained.

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He's repented

The most recent issue of *Scientia Sinica* contains the following correction slip: In the aricle "Devote Every Effort to Running Successfully Socialist Research Institutes of Science" (*Sci. Sin.*, XIX, No. 5), "the arch unrepentant capitalist-roader in the Party Teng Hsiao-ping" should read "Teng Hsiao-ping".

news and views

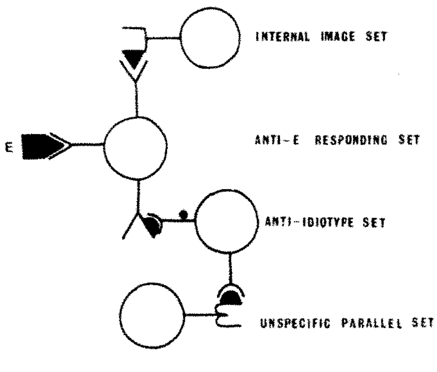
Immunological networks

from Martin Raff

SEVERAL years ago, Jerne proposed a network theory of the immune system[1,2] and predicted that, if correct, its impact on immunology could be as great as that of the selective theories of antibody formation[2]. Although crucial information is still lacking, increasing evidence suggests that he may be right.

Idiotype networks

Antibodies not only recognise antigens, they themselves can act as antigens. Those antigenic determinants (or epitopes) that are related to the antigen-combining site of an antibody are called 'idiotypes'. Jerne suggests that idiotypes are more than just useful tools for immunologists—they may form the basis of a complex immunological network which is central to the functioning of the immune system. He argues as follows. Since the repertoire of antigen-binding sites (and thus idiotypes) in an animal is very large (perhaps $\sim 10^6$), no one idiotypic determinant, in the absence of antigenic stimulation, is present in high enough concentration to induce tolerance. Thus in the course of an immune reaction the increased concentration of specific idiotypes would stimulate anti-idiotype immune responses. Such anti-idiotype (and presumably, in turn, anti-anti-idiotype) responses would play an important part in regulating an immune response. It is known that animals can be stimulated to make antibodies to the idiotypic determinants of antibody molecules produced by other animals of the same species or strain, or even of their own antibodies (see below); and this suggests that an animal possesses antigen-binding sites that can recognise any idiotype occurring in the species. This in turn suggests that within an immune system, any idiotype can be recognised by a set of antigen-binding sites and any antigen-binding site will recognise a set of idiotypes.



JERNE'S IDIOTYPE NETWORK

Thus, a lymphocyte clone, with Ig receptors having an antigen-binding site for epitope E and bearing a characteristic set of idiotype I, can in principle form a functional network with two other sets of lymphocytes (see diagram)—idiotype-reactive cells with receptors having binding sites for I, and cells with Ig receptors bearing idiotypes which are cross reactive with E and thus recognised by the anti-E lymphocyte clone. Jerne calls the latter set the "internal image" set of lymphocytes. A fourth set of cells in this interacting network might consist of cells with Ig receptors bearing I idiotypic determinants but having antigen-binding sites for epitopes other than E. In addition, each of these three lymphocyte sets, which are functionally linked by idiotype to the original anti-E lymphocyte clone, forms its own interacting network with other lymphocytes, also built around the idiotypes of their Ig receptors. Assuming that both T and B lymphocytes share at least some idiotypes[3–5], both classes of lymphocyte would participate in such a functional network, which is in a dynamic steady state with the elements interacting both positively and negatively. Jerne tentatively proposes that the anti-idiotype (anti-I) set of lymphocytes may have an inhibitory effect while the "internal image" set may have a stimulatory effect on the anti-E clone, the overall effect being a "balanced suppression" which must be overcome in order to induce an immune response to E (ref. 2). However, it may be that within each set both stimulatory and suppressive lymphocyte subpopulations are represented.

The immune system is thus visualised as a complex network of antigen-binding sites that recognise sets of idiotypes, and of idiotypes that are recognised by sets of antigen-binding sites. Its response to antigen is, therefore, best understood in terms of a reverberating perturbation of the network, rather than as a response of individual antigen-sensitive lymphocytes.

Many of the elements required for such an idiotype-based network have now been shown to exist. T (refs 6–8) and B (refs 8–17) lymphocytes reactive to idiotypes present on the animal's own Ig molecules have been unequivocally demonstrated. Anti-idiotypic antibodies, administered exogenously[3–5,7,18–20] or produced endogenously[16,17,21] have been shown to suppress[3–5,7,18–19,21] or stimulate[4,20] T and/or B lymphocytes which share the relevant idiotypes, and anti-idiotype-reactive T cells have been shown to suppress the production of antibodies carrying the idiotype[7]. Moreover, it has been demonstrated that the antibody response to phosphorylcholine in BALB/c mice, which is largely restricted to the response of a single clone carrying the TEPC 15 idiotype[22], is accompanied by the production of anti-idiotype (anti-TEPC 15) antibodies[13,15]. Therefore, idiotype-bearing and complementary anti-idiotype reactive lymphocytes coexist in the immune system and, in at least one case of a clonally restricted antibody response[13–15], both sets of lymphocytes are activated during an immune response to an appropriate antigen. What remains to be demonstrated is that such autogenous anti-idiotype reactions occur in the more common situation where the initial response is

Volcano spacing in East Africa

from Peter J. Smith

THE near-rectilinear pattern of volcanoes in the Galapagos Islands was first noticed by Darwin (in *Geological Observations on Volcanic Islands*, London, 1891) and the near-uniform spacing of Hawaiian volcanoes was first observed by Green (in *Vestiges of the Molten Globe*, Honolulu, 1887). Since then, numerous workers have claimed to detect regularities in volcano siting, and some have pointed out that the spacings of oceanic volcanoes in particular appear to be roughly equal to the thickness of the local crust. More systematic attempts to relate volcano spacing to crustal thickness were doomed to failure, however, because (as we now know) the crust is not the correct unit to consider in this context.

Interest in the problem of volcanic intervals therefore largely died out until in 1974 Vogt (*Earth planet. Sci. Lett.* **21**, 235; 1974) revived it in the light of modern ideas on Earth structure. Vogt found that within oceanic provinces volcano spacings are indeed remarkably uniform. In 'continental' subduction zones volcanoes are typically 70 km apart whereas in island arcs they are 55 km apart. The separations of volcanoes associated with 'hot spots', on the other hand, increase with the age of the surrounding oceanic crust. But what, figuratively speaking, links all these volcanoes is the thickness not of the crust but of the lithosphere. Thus Vogt was able to show that within any given volcanic province the distance between adjacent volcanoes is roughly equal to the thickness of the local lithosphere.

But is this also true for continental volcanism? To find out, Mohr and Wood (*Earth planet. Sci. Lett.* **33**, 126; 1976) have examined volcanoes along the northern section of the East African rift (roughly 15° N–5° S). This is a particularly suitable area because, although data are fewer, lithospheric thicknesses are generally better determined than in oceanic zones and are known to cover a much wider range. In any event, there are sufficient data to allow Mohr and Wood to show convincingly that the volcano spacing = lithospheric thickness relationship holds good for East Africa as well and is independent of the type of volcanic rock involved.

Why the relationship holds, apparently universally, is less clear, however. Vogt made two suggestions: first, that a volcano taps all magma within a given radius (a 'scavenging radius') related to lithospheric thickness through a conical volcanic conduit of fixed angle (25°) and, second, that volcanic sites are controlled by lithospheric fracture grids with spacings related to lithospheric thickness. He favoured the latter mechanism, though leaving open the question of whether the grids predate, or are contemporaneous with, the magmatic activity. By contrast, in a situation in which the crust is exposed Mohr and Wood find no evidence at all of volcanic control by pre-existing fracture grids, although again the possibility of contemporaneous rifting remains open.

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clonally heterogeneous, and that they have an important immunoregulatory role. *A priori* it seems highly likely that they do.

Moreover, it makes sense that just as environmental antigens stimulate and recruit relevant T and B cell clones into the recirculating, long-lived lymphocyte pool, the antibodies and receptor-bearing cells produced in the course of these responses should stimulate and recruit anti-idiotype-reactive clones into the pool; thus, the immune system would organise itself into an idiotype-based network. It should not be difficult to establish that such a network is important in immunoregulation: if eliminating anti-idiotype-reactive T and B cells from a cell population markedly alters the response of the idiotype-bearing clones to the appropriate antigen, the point would be made. From a practical standpoint, the TEPC 15 idiotype response to phosphorylcholine[8,13,15,18] would be an attractive system for such an experiment, but ultimately it will be important to demonstrate an effect in a non-restricted, clonally heterogeneous response.

As Jerne has pointed out[2], the major weakness in an immune network theory at the moment is the incompleteness of our knowledge of the component parts (immunologists are still subdividing T and B cells into an increasing number of subclasses, and the end is not yet in sight) and our ignorance of the mechanisms involved in positive and negative interactions between the parts. The task is clear, and it seems unlikely that mathematical model-building[22,23] will be of great value until more information about the elements in the network is available. In the meantime, the lack of detail makes it possible to use the network theory to explain various immunological paradoxes that are difficult to explain in other ways[2]. For example, it provides, in principle, a way out of the Oudin-Cazenave enigma[24], which is the observation that antibodies produced against two non-cross-reacting epitopes of one antigen molecule may have similar idiotypic determinants. It can also explain another of Oudin's paradoxical observations[25]—that immunisation results in the production of nonspecific Ig molecules bearing the same idiotypes as the specific antibodies; in fact, this suggests the existence of the "unspecific parallel set" of lymphocytes in Jerne's network (see Fig.).

An allotype network

The dramatic observations of the Herzenbergs and their colleagues[26] on chronic allotype suppression have uncovered another type of lymphocyte network, based on allotype rather than idiotype. Allotypes are alloantigenic epitopes on Ig molecules which are unrelated to the antigen binding site and have no known functional significance. In [SJL(Ig-1b)×BALB/c (Ig-1a)]F_1 mice, perinatal exposure to antibodies directed against Ig-1b allotype determinants (present on IgG molecules) results in the generation of suppressor T cells which specifically inhibit the production of all antibodies bearing the Ig-1b allotype but not antibodies bearing the other parental allotype (Ig-1a). Herzenberg *et al.*[26] have now shown that the suppressor T cells in these allotype-suppressed mice do not directly act on Ig-1b B cells, as originally assumed; rather, they suppress a specific class of helper T cells which normally help Ig-1b B cells to differentiate into Ig-1b antibody-secreting cells. Besides indicating that T cell suppression of B cell responses may act indirectly by way of T helper cells, as is probably also the case in carrier-specific T cell suppression[27], these striking experiments demonstrate that, in these mice at least, there are allotype-specific T helper cells, which help only B cells bearing the Ig-1b allotype, and suppressor T cells which specifically suppress these allotype-specific T helper cells. These three sets of cells constitute a lymphocyte network organised around allotype, which, unlike an idiotype-based network, is independent of antigen specificity and would therefore include many lymphocyte sets functionally linked in an idiotype network.

There are however difficulties in inducing chronic allotype suppression using other mouse strain combinations[28], and it is not clear whether allotype networks are the exception or the rule. Nor is it obvious what purpose they serve. Since it is not known whether allotypes have any functional significance one cannot assess the value of regulating immune responses on the basis of allotype. On the other hand, there would be obvious advantages in regulating immune responses on the basis of Ig class. At the moment, the only hint of lymphocyte networks based on Ig class is the report of T helper cells that are specific for IgE B cells[29]. It is possible that the finding of an allotype-based network will point the way to an eventual demonstration of similar lymphocyte networks based on Ig class. In view of the increasing evidence for Ia-bearing molecules with immunoregulatory function, and Ia-bearing cell-surface acceptors for these molecules (see ref. 30), it is also conceivable that there may be lymphocyte networks organised around determinants of the major histocompatibility complex (MHC), just as there are around Ig epitopes.

There are other intriguing questions raised by the demonstration of an allotype-based network[26]. How do the cells in the network recognise each other? The conservative answer would be by the use of allotype-bearing and allotype-recognising antibody molecules. But it has been difficult to demonstrate Ig allotypes on T cells; and the relatively high concentrations of allotype in the serum might be expected to block allotype-reactive receptors on cells. What is the relationship between carrier-reactive and allotype-reactive T helper cells? Apparently, both can be required to activate a B cell, but are they the same cell, with one receptor for allotype and one for carrier? Since evidence is growing that most T cells preferentially respond to antigen in association with MHC determinants (see ref. 30), is it possible that helper T cells have separate receptors for carrier, allotype (and/or Ig class) and MHC antigens? ☐

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Ultrasonic assessment of arterial pathways

from C. D. Field

NON-invasive determination of blood vessel function by measurement of Doppler shifted ultrasound reflected from moving red cells is rapidly gaining clinical acceptance as a useful tool in screening for vascular disease. Abnormal patterns in the reflected Doppler-shifted signals compared with those obtained from normal healthy individuals are beginning to be characterised and correlated with various clinical conditions, as defined by conventional radiography and other techniques, and to be used diagnostically. For routine screening, ultrasound has several important advantages over conventional radiography. Injections of toxic dyes are not needed to locate the vessel under study, and because power levels are so low (about $30\ \mathrm{mW\ cm^{-2}}$) measurements can be repeated as often as required.

To detect red cell velocities by Doppler shifted ultrasound two identical piezoelectric crystals are mounted coplanar at an angle to the vessel under study, as transmitter and receiver. Activation of the transmitting crystal produces a narrow beam of ultrasound which is coupled into the tissue by way of a water-based gel. Activation frequencies vary from 1 to 10 MHz and their physical properties are well documented (Gosling and King, *Cardiovascular Applications of Ultrasound*, North Holland, 1973). Incident radiation returns to the receiver from the tissue both by reflection from large surfaces and reradiation by any small elastic particles in the beam path. Donald (*Brit. J. Radiol.* **49**, 306; 1976) has recently discussed the use of reflected radiation from structures of differing acoustic impedance and Gosling (*IEE Medical Electronics Monographs* **21**, 73; 1976) has considered the use that can be made of the backscattered Doppler shift signals from moving blood in arteries.

When a blood vessel is bathed in an ultrasonic beam a wide spectrum of Doppler-shifted frequencies is backscattered from vessel to receiver at each instant of time, because velocities of the red cells vary across the lumen. This spectrum varies over the cardiac cycle and, with beam frequencies of 1 to 10 MHz, is in the audio range for both healthy and diseased arteries. All signals received from a vessel can be displayed as a sonagram—the Doppler shifted frequencies along the Y axis, time along X and blackness of trace proportional to signal amplitude. The striking aspect of sonagrams from any vessel is their well-defined maximum frequency envelope over the cardiac cycle which can be used to characterise an arterial site under specified conditions.

Although certain direct physiological information can be obtained from a single sonagram a great deal more information can be gained by using two simultaneous observation sites at consecutively paired positions along the vascular tree. Phasic changes in such paired sonagrams are easily recognised as 'normal' or abnormal. These changes may be due to trauma, congenital defect or pathological change and the observed differences from normal may be correlated empirically with clinical condition and used diagnostically.

As well as arterial status, functional ability of the heart can be assessed. This is done with one Doppler detector over a neck artery, a second on the chest plus an ECG and a heart microphone. A simultaneous four line trace shows all the cardiac intervals (Gosling, Beard and Pickup, in preparation).

No Doppler shift ultrasound volume flowmeter is yet available for clinical use. However, it has been realised that the shape of the flow-velocity pulse at any arterial site, and hence the flow-velocity sonagram, carries information about the whole cardiovascular tree. Baskett *et al.* (*Cardiovascular Res.* 1977 in the press) has used this approach to estimate the extent of disease in the internal carotid artery supplying the brain (a common source of strokes). An intrinsic property of an artery wall is its compliance. This is defined as fractional change of lumen diameter per 10 mmHg pressure and so may be compared in subjects with differing pulse pressures. Gosling (*op. cit.*) considers that it may be a useful indication of vascular disease and that *in vivo* measurement of this parameter could lead to earlier detection of abnormalities. There is some difficulty in measuring compliance directly but it has been demonstrated that in certain conditions the average value of compliance for any length of artery is given by a simple function of the delay time between entry and exit pulse waves, that is, sonagrams, for the same heart beat and their distance apart on the vessel. This indirect measure of compliance is easily made through the skin, and amounts to determination of the apparent propagation speed of the flow velocity pulse.

The Non-Invasive Angiology Group at Guy's Hospital Medical School, London, has recently applied this pulse wave velocity approach in a study of compliance in aorta, pelvic arteries and leg arteries. They find that aortic compliance in normal subjects increases by some 70% in the first decade for both males and females, decreases to birth value over the next decade and then only slowly decreases over the rest of the life span to about half-birth values in old age. Their results also show that the female diverges from the male at menarche. Women retain a significantly higher aortic compliance until menopause, after which there is again no difference. In patients with diabetes it was found that insulin-dependent juveniles (diagnosed less than one year before measurement) had abnormally high aortic compliance, but that older patients (over 40 years) whose diabetes was controllable by diet exhibited normal or lower values. These results are markedly different from the accepted view that diabetic children and adults always have stiffer arteries than normal. That physiological function may affect structure has recently been demonstrated by Berry *et al.* (*Brit. Heart. J.* **38**, 510; 1976) who showed that compliance of the aortic-iliac pathway is anomalously high in those children born with a single umbilical artery. Such compliance studies as these clearly have implications in the search for causal factors of arterial occlusive disease, the biggest single cause of death in society today.

It may be concluded that Doppler-shifted signals from various arterial sites and pathways of the body can yield much useful physiological and pathological information. Research teams such as those at Guy's Hospital are now able to scan, non-invasively, specific arterial segments (or pathways) and objectively decide whether they are normal or not. Furthermore, this is being done on an out-patient basis in the relaxed unsedated patient. Although still in their infancy as clinical tools, these techniques and methodology offer a complementary approach to conventional methods with obvious advantages to clinicians and patients alike. □

Diamond platelets are interstitial carbon

from John Walker

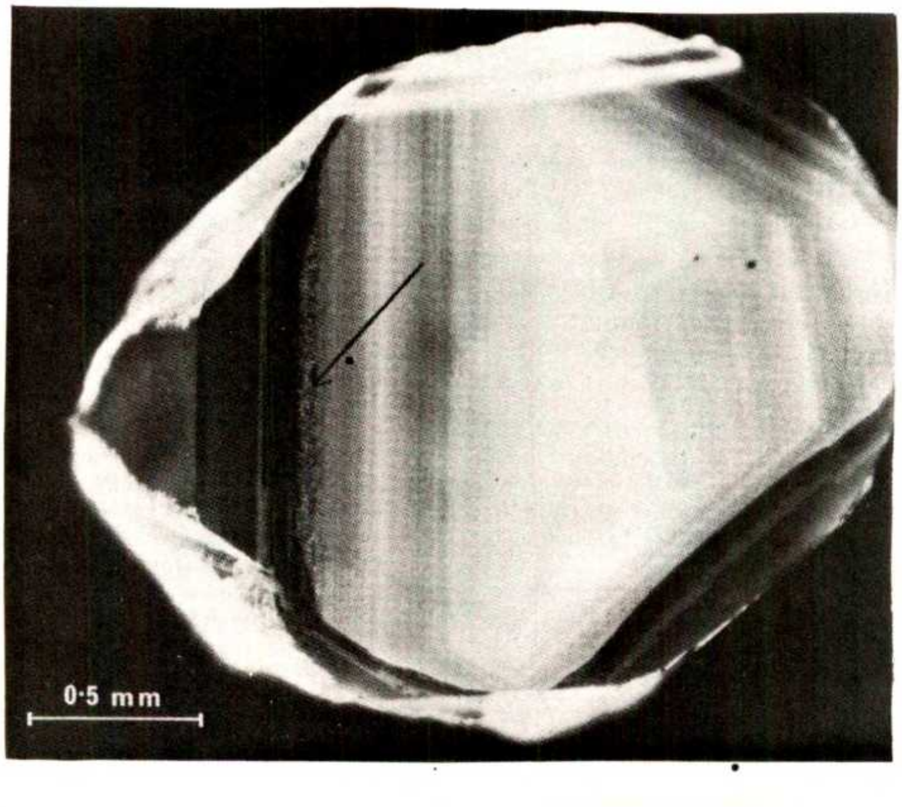
IN a recent *News and Views* item (*Nature*, **264**, 317; 1976) I noted in passing that we were still ignorant of the nature of one of the defects found in diamond—the so-called 'platelets'. This ignorance has now been rectified by G. S. Woods (*Phil. Mag.* **34**, 993–1012; 1976). The platelets (large planar defects on {100} planes) are apparently composed of carbon atoms in interstitial positions (that is, squeezed into the empty interstices between the normal lattice sites).

An anomaly in the X-ray diffraction photographs of some diamonds was first noted by Raman and Nilakantan in 1940. The normal diffraction photograph contains a symmetrical array of spots corresponding to reflection of the X-rays from particular planes of atoms. But in some diamonds—what we now call type Ia—the spots were accompanied by streaks ('spikes'). In 1956 Frank (*Proc. R. Soc. Lond.*, **A237**, 168; 1956) proposed that the streaks were due to planar aggregates of foreign atoms, oriented along the {100} planes—the platelets. His prediction was confirmed by Evans and Phaal in 1962 (*Proc. R. Soc. Lond.* **A270**, 538) when they observed the platelets directly using electron microscopy.

Frank had proposed that the foreign atoms were silicon. Subsequently analyses showed that this element was an uncommon impurity in diamond, whereas concentrations up to $\sim 12\%$ were required to account for the platelets. When Kaiser and Bond (*Phys. Rev.* **115**, 857; 1959) showed that type Ia diamonds contained nitrogen in the required concentration, the obvious conclusion was that the platelets were made of nitrogen—obvious, but wrong.

In 1969 Sobolev, Lisoivan and Lenskaya (*Sov. Phys. Dokl.* **12**, 665) showed that the platelet concentration, as measured by the X-ray spike intensity, did not correlate with the total nitrogen concentration as measured by the infrared absorption peak at $7.8\ \mu m$. The platelets could contain little if any nitrogen.

These results, though controversial, were soon confirmed by a completely independent technique—thermal conductivity. The way the thermal conductivity of a crystal varies with temperature depends on the size and concentration of any defects in it. Turk and Klemens (*Phys. Rev.* **B9**, 4422,



Cathodoluminescence pattern from a diamond. The 'giant' platelets are individually resolvable as white dots in the arrowed band.

1974) showed by this method that at least 97% of the nitrogen in the diamonds they studied was present in aggregates smaller than one nanometre in diameter. No more than 5% could be in the platelets, which are typically 50 nm across.

So what could the platelets be made of? Diamonds had been analysed by many different techniques and no other impurity had been found in anything like the proportions necessary. Evans (*Diamond Res.* page 6, 1973) therefore proposed that they were carbon atoms; but carbon atoms arranged in such a way as to break the normal periodic stacking sequence of the diamond lattice—a plane of interstitial carbon atoms. Woods's paper confirms Evans's hypothesis.

Woods managed to find diamonds containing 'giant' platelets up to 5,000 nm in diameter (see diagram) which he then studied by electron microscopy. He confirmed that these were just large examples of the normal platelets by observing in one of his specimens a continuous gradation in size, down to the more usual 50 nm. Accompanying the giant platelets were loops of perfect dislocation, of interstitial character (like the platelets), and also lying in {100} planes. Their shapes, dimensions and geometries were so unusual, and so similar to the platelets, that Woods concluded they were produced by the platelets. (The alternative possibility, that they produced the platelets, seemed unlikely because the platelets were sometimes present when the loops were not, whereas the converse was never observed). Some of the dislocation loops were L-shaped—they changed from one to another non-parallel {100} plane. Woods therefore proposed that they were created by the interaction of two perpendicular platelets. When the two grew large enough to touch, or came sufficiently close to feel each others' strain fields, two dislocation loops would be created (or a single L-shaped loop if the two loops were in contact). The crucially important point is that, given the geometry of the platelets, of the loops, and of the diamond lattice, and bearing in mind that the platelets are interstitial in character, the platelets can create the loops only if the platelets themselves are composed of carbon atoms in interstitial sites.

If Woods's interpretation is correct —and it is certainly consistent with all the extant data—then he has solved a puzzle that has fascinated diamond physicists for years.

As usual, however, there are questions that are still unanswered. Why is it that platelets are seen only in diamonds containing nitrogen aggregates? Are the platelets formed by interstitial nitrogen atoms displacing substitutional carbon, as Evans suggested? How exactly do the platelet atoms bond to the rest of the lattice? Why are platelets never seen in synthetic diamonds containing nitrogen? Do the platelets play any part in diamond synthesis, or are they merely the product of ageing in diamonds containing nitrogen? Woods's paper is likely to stimulate new thinking on all these questions. □

Exploiting exotic plants

from Robert M. May

ALTHOUGH more than 3,000 plant species have been utilised by man for food, only about 20 major crops today provide food for most of the world.

Motivated by the thought that "to help feed, clothe, and house a rapidly increasing world population, it is timely to consider neglected or little-known plant species", a panel of the National Research Council of the US National Academy of Sciences has presented a report on *Underexploited Tropical Plants with Promising Economic Value* (published by the Board on Science and Technology for International Development, NRC, 1975: see also the excellent summary by Langford, *NAS News Report*, **26** (8), 1; 1976).

The report begins by noting that, in tropical countries, concentration on a small number of major food crops stems not from their inherent superiority, but rather from historical accident. "Many indigenous species may possess equal merit, but were disregarded during the colonial era when consumer demands in European countries largely determined the cultivation (and research) priorities in tropical agriculture". In addition, "most agricultural scientists are unaware of the scope and potential offered by tropical botany. The discipline suffers largely because the major centres of scientific research are located in temperate zones" (NRC, *op. cit.*).

Reliance on a small number of plant species carries risk, because monocultures are vulnerable to catastrophic failure brought about by disease or climatic fluctuations. But, due to the sprawl of cities, the high cost of maintaining botanic gardens and habitat reserves, and the spread of conventional agriculture, potentially useful plants (including varieties necessary for genetic diversity in the development of new food crops) are being destroyed, and some are "in imminent danger of extinction". In urgently recommending programmes of systematic collection and study of tropical flora, the NRC panel hopes that many underexploited plants may thereby follow the example set by the soybean, which has become a staple in many parts of the world only within the past 50 years.

The report describes 36 plants, selected from among 400 nominated by plant experts in many countries. For each of these 36, the report indicates the advantages and limitations of the plant, and outlines special requirements and research needs.

A prime example is quinua. This tall, hardy herb of the Andean altiplano was a staple in the Inca empire. Its grain is rich in protein, with a good balance of amino acids; it is possibly a better protein source than most of the true cereals. Its disadvantage is a bitter taste, derived from saponins in the outer seed layer. The sort of questions that arise for quinua are fairly typical: can it be grown at lower altitudes and in warmer climates; can a satisfactorily saponin-free variety be produced; or should the saponin be retained as a natural defence against pests, and be milled out after harvesting?

A native palm of the Amazon region, *Jessenia polycarpa*, bears fruit clusters from which can be extracted a food oil akin to olive oil. None of the several *Jessenia* species has been cultivated commercially. To quote Langford, "that no one knows how many years are required for *Jessenia* to mature and bear seed indicates the extent of research needs pertaining to many tropical plants".

Naranjilla, "the golden fruit of the Andes", is little known outside Colombia and Equador, where it is used to flavour confections and preserves, and to make a popular fruit drink. The plant could apparently flourish, and be put to these uses, throughout the African and Asian tropics.

In Chile's salt-covered Atacama desert, the leguminous plant tamarugo not merely survives, but can sustain grazing sheep at a density approaching that for green pasture. The report observes that since 1968 more than 5,000 sheep have been raised on tamarugo plantations, and recommends that trials be undertaken in other parts of Latin America, the Middle East, and in arid Africa.

Turning from food and forage crops, the report discusses, for example, guayule. This desert shrub, found in Mexico and the southwestern USA, produces a latex which, when refined, is similar to the rubber obtained from *Hevea* trees in southeast Asia.

The report emphasises that all such

novel plants should be seen as complements to, not as substitutes for, conventional tropical crops. It also acknowledges that the problems of exploiting new plants are not just horticultural, but are economic and sociological: new crops depend on the creation of new markets. ☐

Nucleon coalescence

from P. E. Hodgson

RECENT experiments on the interaction of relativistic heavy ions with silver and uranium have provided evidence that the emitted nucleons tend to stick together to form composite particles, so that the numbers of high energy ^{3}He and ^{4}He particles are two or three orders of magnitude greater than those found for proton-induced reactions at comparable velocities. It was found that more ^{3}He were emitted than ^{4}He, and this made it doubtful whether both their emission could be explained by the same process.

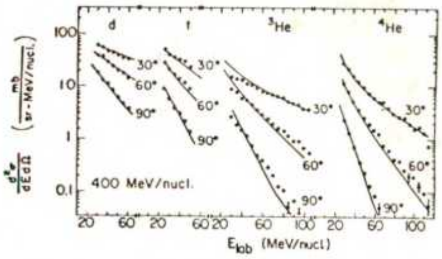
To study this matter in more detail, Gutbrod and colleagues (*Phys. Rev. Lett.*, **37**, 667; 1976) have measured the energy spectra at several angles of the protons, deuterons, tritons, helions (^{3}He) and alpha particles (^{4}He) emitted when uranium is bombarded with 250, 400 and 2,100 MeV per nucleon ^{20}Ne ions and with 400 MeV per nucleon alpha particles, and some typical results are shown in the figure.

To try to account for these distributions, they developed a theory that had already been used by Butler and Pearson to explain the observation of high energy deuterons emitted from nuclei bombarded with energetic protons. This theory assumes that among the cascade of nucleons emitted from such reactions there will be some neutron–proton pairs with rather low relative momenta. The nucleons of each pair can interact with each other and with the surrounding nuclear field to form a deuteron, the nuclear field serving to absorb excess energy and momenta. This model enables the energy spectra of the deuterons to be calculated from that of the cascade nucleons.

The theory was modified to calculate the spectra of the lighter particles emitted from relativistic heavy ion collisions, and the results are compared in the figure with the experimental data. It is seen that the overall features are accounted for very well.

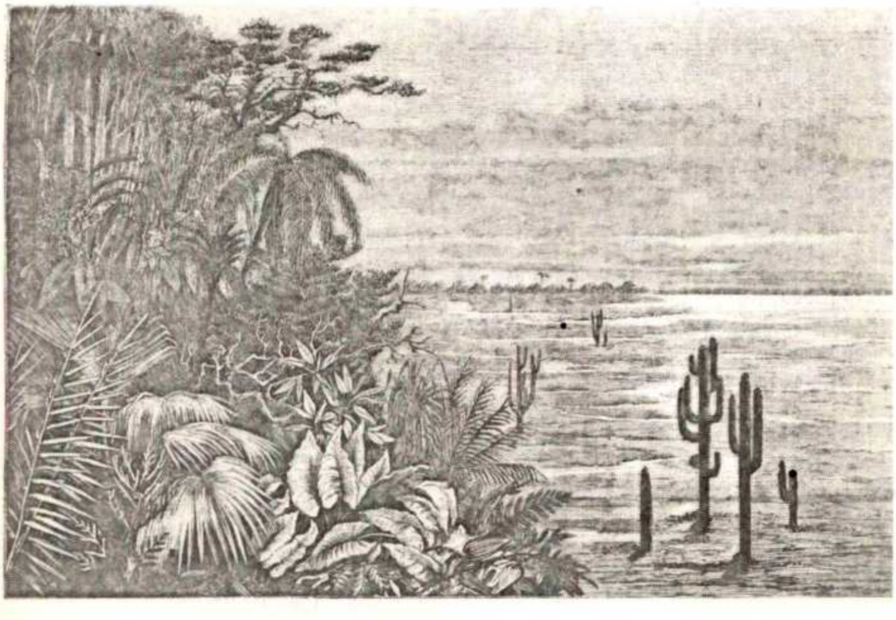
An important parameter of the theory is the greatest relative momentum between the neutron and the proton for which they can coalesce. This momentum was adjusted for each of the reactions investigated so as to give the best overall fit to the experimental data, and it was found that the best values were around 130 MeV/*c* in each case. This constancy checks the validity of the theory.

It still remains to understand the details of the mechanism of nucleon coalescence, but it is certainly encouraging that a relatively simple theory can account so well for so much experimental data relating to very complicated reactions.



Experimental energy spectra of the deuterons (d), tritons (t), helions (^{3}He) and alpha particles (^{4}He) emitted from uranium irradiated by ^{20}Ne ions at 400 MeV per nucleon.

A hundred years ago



TROPICAL FORESTS IN HAMPSHIRE. The Valley of the Bourne restored to represent the conditions which are supposed to have existed during the deposition of the Lower Bagshot Formation: Looking at the scene from a southern standpoint we should see to the north the distant chalk range. Whilst along the shore of the opposite bank of the valley we could with some difficulty detect the various forms of vegetation, which we should see with greater clearness in the more immediate foreground. In this valley a singular stillness must have prevailed, as no trace of animal life whatever has been found, except a feather and a few insect wings blown in from the southern bank.

Of the following at least we are pretty sure, and of numerous others we can be almost sure, but there are indications of very many besides, the relationships of which are at present but imperfectly defined.

Here we should see the graceful fan-palm and the feather palms, adding softness to the view by their elegantly-curved and drooping leaves, laurel and dwarfed oak, stately beeches, clumps of feathery acacia, trellised and festooned with smilax, the trailing aroid, with its large and glossy foliage and an undergrowth of *Mimosa* and of cypress in the swampier ground, and variations in colour caused by the foliage of cinnamon and fig, and the ground clothed with ferns and sedges. On the barren sands of the distant valley are growing clumps of giant and weird-looking cactus. It is not difficult to picture to ourselves the view.

All this beauty is gone. We have nothing but these records of what must have been a view of great loveliness, which only the toil of the geologist can even faintly reproduce.

From *Nature*, **15**, January 18, 261; 1877.

review article

Homoeotic genes, compartments and cell determination in *Drosophila*

Gines Morata† & Peter A. Lawrence

Normal development of Drosophila *depends on the activity of certain 'master' genes. We review recent work which suggests that these genes control the developmental pathways taken by specific groups of cells as they divide and construct precisely defined parts of the adult fly.*

EVERYDAY experience tells us that the form of an adult animal is defined in great detail by its genotype. An example is an individual human face which, although each half develops separately, displays almost perfect bilateral symmetry. Yet how genes determine detailed structure, shape and size is unknown. We know more about the genetics of *Drosophila* than any other multicellular animal and yet even here we do not understand how genes control the development of any organ. If we want to unravel the genetic logic of development we could start with *Drosophila* and try to classify genes into those with a controlling role and those with a more subsidiary executive function.

The best candidates for controlling elements are the so-called homoeotic genes. Mutations in these genes characteristically transform one body part into another. They are particularly well studied in *Drosophila* but they have been found in some other insects[1,2]. In essence the homoeotic transformations are replacements of normal structures by other normal structures which in wild-type flies are found elsewhere. The mutant *bithorax* is a good example of a homoeotic transformation; wildtype flies have a pair of wings and a pair of halteres (Fig. 1) but mutations in the *bithorax* gene[3] transform the anterior half of the haltere into anterior wing. The wing tissue formed in the haltere in these mutant flies is very similar in all respects to normal wing tissue. The wild-type function specified by the *bithorax* gene is thus required during growth of the anterior cells of the haltere; if the function fails these cells will develop as in a wing. The mere existence of such a mutation indicates that there is at least one moment in normal development of the haltere in which the alternative—haltere or wing—is open. In this feature lies the current interest in homoeotic genes; they are single loci which determine the developmental pathways taken by groups of cells.

It has recently been discovered, in *Drosophila*, that special groups of cells are set aside at early stages of development, and that the cellular descendants of these groups form specific regions in the adult called 'compartments'[4,5]. In this review we discuss the evidence that the development of compartments is under the control of homoeotic genes, and highlight the relationship between this new hypothesis[4], and the older concept of cell determination. Since one might expect similar types of genetic control of development in different groups of multicellular animals, we also briefly consider whether these phenomena occur in vertebrates.

In *Drosophila* the cuticular parts of the adult fly are constructed during the pupal period, from a set of 'imaginal disks' which form in the embryo and grow during the three larval stages: for example the wing imaginal disk forms the wing itself as well as those dorsal thoracic parts belonging to the wing segment. Most of the homoeotic mutants produce intersegmental transformations, the structures formed normally being found in another disk. Thus the *bithorax* mutation transforms the haltere segment into part of the wing segment (Fig. 1), *spineless-aristapedia* transforms the antenna into leg (Fig. 1), and *opthalmoptera*, the eye into wing. There are at least two mutants that produce intrasegmental transformations where one part of the disk is the duplication of a different part of the same disk: the mutation *engrailed* transforms the posterior structures of wings into the corresponding anterior structures[6] (Fig. 1). In *wingless* flies the wings are replaced by a mirror image duplication of the dorsal thorax[7,8].

In those cases where homoeotic mutations affect more than one disk they produce homologous transformations in different disks. For example, in *engrailed* posterior wing is transformed into anterior wing, posterior leg into anterior leg, and, probably, posterior haltere into anterior haltere. *bithorax* transforms not only the anterior haltere into anterior wing, but also, the anterior parts of the legs of the haltere segment into the anterior parts of the legs of the wing segment. Clearly there is a common requirement for the wild-type product of the *bithorax* gene in these two regions of the haltere segment, even though the cuticular structures produced by these two groups of cells are distinct. By contrast the type of cuticle in the anterior haltere is almost indistinguishable from that of the posterior haltere, yet the anterior disk cells which form the haltere are sensitive to the *bithorax* mutation while the posterior disk cells are not. Thus the function of homoeotic genes seems to be localised to particular regions of the body.

We need to define as exactly as possible the realms of action of the homoeotic genes. Unfortunately most mutations produce transformations with variable penetrance (penetrance = percentage of the mutant flies which show the phenotypic trait) and variable expressivity (expressivity = degree to which the mutant phenotype is shown by a given fly) which makes it difficult to define the limit of activity of the wild-type genes. Moreover, the expressivity

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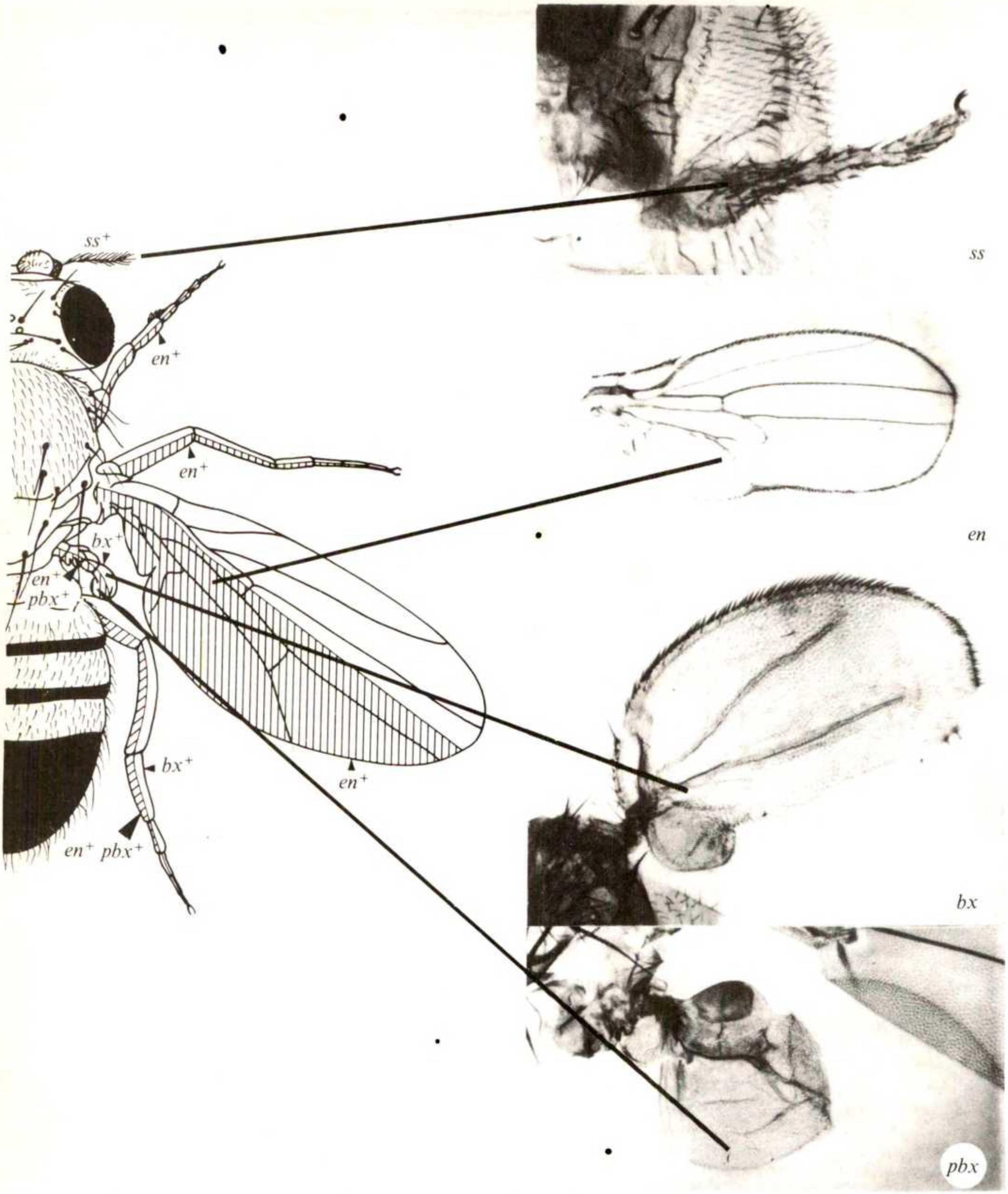

Fig 1. Homoeotic transformations produced by the mutants *spineless-aristapedia* (*ss*), *engrailed* (*en*), *bithorax* (*bx*) and *postbithorax* (*pbx*). A diagram of a wild-type fly is on the left. On the right there are photographs of some of the transformations produced by these mutants. *ss*: The antenna is transformed into leg. Mutations in this locus produce an effect in the antenna only. *en*: the posterior wing is partially transformed into anterior wing as witnessed by the effects on the veins[6,17] and the posterior row of "anterior" bristles. This mutation also transforms the posterior legs into anterior and, probably, posterior haltere into anterior haltere. Consequently the activity of en^+ is required in the posterior regions of at least these structures (shaded in the diagram of the wild-type fly). *bx*: the anterior haltere is transformed into anterior wing and also the anterior III leg is transformed into anterior II leg (this transformation can be recognised because the II and III legs are not identical, each having distinctive cuticular markers). The genotype of the transformation shown in the picture is $bx^3/Df(3R)bxd^{100}$ where $Df(3R)bxd^{100}$ is a chromosome in which the *bithorax* gene is deleted[15]. For this reason the phenotype is very extreme. Notice the sharp boundary between the transformed anterior wing and the unaffected posterior haltere. Only the anterior haltere and anterior III leg require the activity of bx^+. *pbx*: The posterior haltere is transformed into posterior wing and also the posterior III leg is transformed into posterior II leg (as in the *bx* transformation this can also be recognised by cuticular markers). The genotype of the transformation shown in the picture is $pbx/Dp(3;3)bdx^{100}\ Df(3R)P115$ where $Dp(3;3)bxd^{100}\ Df(3R)P115$ is a chromosome in which the *postbithorax* gene is deleted[15]. For this reason the phenotype is very extreme. Notice again the sharp boundary between the unaffected anterior haltere and the transformed posterior wing. Only the posterior haltere and the posterior III leg require the activity of pbx^+.

of some of those mutants varies greatly in different regions of the same disk[9]. The probable reason for these incomplete transformations is that the mutants are leaky (*hypomorphs* in the *Drosophila* terminology) in which some wild-type function remains. Mutants like *Antennapedia* of *Rappaport* (Ant^R) produce a partial transformation of arista and antenna into leg so that the final structure is a mixture of phenotypically wild-type (arista or antenna) and mutant (leg) cells[10]. This is also the case for other mutants such as the different alleles of *spineless-aristapedia*[11] and *opthalmoptera*[12] and some *bithorax* alleles. According to the description of *Antennapedia*[10], it only affects the antenna part of the eye-antenna disk (even some antennal disk derivatives like rostralhaut and maxillary palpus are not affected) whereas *Nasobemia* which is probably in the same locus[13] very often transforms part of the head cuticle belonging to the eye part of the disk[14]. It is conceivable that a very extreme *Antennapedia* mutation could transform the eye and perhaps other structures as well. For these reasons all homoeotic genes should ideally be studied with mutations that completely lack wildtype function—for instance with chromosomes that are deficient for the gene itself. Unfortunately this is usually impractical; the deficiencies are nearly always lethal when homozygous (we know that most deficiencies, even the smallest ones, are lethal[15]) and one has to be satisfied with the viable mutants that are most extreme. Even this is difficult, because too few mutant alleles are available (for example, there is only one allele of *engrailed*)—perhaps because homoeotic genes frequently mutate to a lethal form. For *bithorax*, where there are many alleles, a detailed genetic analysis has been done by Lewis[3,16,17]. The mutation bx^3 is the most extreme known[16]; homozygous bx^3/bx^3 flies show a phenotype only slightly less extreme than bx^3/bx^- (where bx^- is the deficiency of the gene) which indicates that bx^3 almost completely lacks function of the *bithorax* gene. Thus in this case we can be reasonably certain that the extent of the phenotype bx^3/bx^- defines the domain affected by the *bithorax* gene.

We clearly need much more genetic analysis of homoeotic mutants, and new methods are now being developed: although some 90% of induced mutations in *Drosophila* are lethal[18], only about 20% of these are cell lethal[19]—the phenotype of those lethal mutants that are viable in individual cells can be studied in clones made homozygous for the mutation[20].

Determination in mature disks

The concept of determination in the imaginal disks of *Drosophila* has been developed by Hadorn and his associates[21] and discussed in detail elsewhere[22]. It refers to the acquisition by a group of cells of a commitment that precisely defines the fate of their descendants. Unlike many other developmental systems, the imaginal disks of *Drosophila* show a clear distinction between determined and differentiated state; one can demonstrate that the imaginal disks are determined to secrete certain regions of the adult cuticle much before this differentiation starts. An imaginal disk or a fragment of imaginal disk is considered to be determined if, when transplanted into the abdomen of a host larva, it differentiates autonomously into the same structures as *in situ*. When this type of experiment is performed on the mature imaginal disks it is found that they are strictly determined; the wing disk only and always forms wing structures, the first leg disk only forms first leg structures and so on. Their state of determination is more restricted than just leg or wing; within one disk, particular regions, when explanted, form specific parts of the adult structures, and in this way consistent fate maps of the mature disks have been constructed[23,24] and in some cases even individual bristles have been located precisely in the disk[25].

The acquisition of a given state of determination seems to correlate with the ability of determined cells to recognise, and associate with, like cells and dissociate from unlike cells[26]. Cells from a given disk or disk-fragment, after dissociation and mixing, remain mixed together and form integrated patterns of differentiated structures. However, cells from different disks or different regions of the same disk will sort out from each other and form separate patterns. In these experiments each type of cell differentiates autonomously according to its disk of origin[26,27].

We have no direct criterion for determination—the term is a purely operational one; determination may be stable in some experimental conditions but unstable in others. Thus, if imaginal disks are cut and the fragments allowed to grow in *in vivo* culture for several days or more, the state of determination can change. For example, groups of cells[28] in growing fragments of leg disks will frequently 'transdetermine' to other cuticular structures such as wing[23,29]. Even the stability of determination of a given fragment depends on the precise experimental conditions: thoracic fragments of the wing disk will only make thorax after extra growth, but if mixed with wing fragments they make both thorax and wings[30]. For these reasons experiments involving a long period of extra growth in *in vivo* culture cannot be used to deduce the state of determination that the initial fragment had *in situ*.

Determination in development

Determination probably begins at around the blastoderm period; if blastoderm cells are dissociated and mixed they will reaggregate specifically with cells that come from the same region of the egg[31]. If individual anterior blastoderm cells are transplanted to the posterior pole of the egg, they will not integrate with the foreign blastoderm cells, and will differentiate anterior structures in the adult[32].

Clonal analysis is a powerful technique which has been applied to early development and can help to pinpoint the establishment of determination. Use is made of cell marker mutants which alter the colour of the cuticle or the shape of bristles in the adult fly but apparently do not affect the course of development. Staged eggs or larvae heterozygous for such a mutant are irradiated with X-rays. At low frequency, somatic recombination occurs in a cell so that one of its daughters becomes homozygous for the mutant, and generates a clone of cells of different genotype. After metamorphosis this clone is seen as a patch of marked cuticle on the adult. This method provides another criterion for determination, if mainly a negative one; when two structures are included in the same clone it shows that the original marked cell was not determined exclusively for either[22]. However, if two structures are never included in the same clone, it does not necessarily mean that their ancestor cells were determined differently. One must then show that the clones are large enough for at least some of them to have included the two structures and also that no physical barrier separates those structures in normal development.

In a careful analysis of early development of imaginal disks, it has been shown that clones produced by irradiation of the embryo at the time of cellular blastoderm can comprise structures of wing and second leg (both belong to the mesothoracic segment) proving that at that time the cells are not exclusively determined for either[33-35]. Clones do not cross from one segment to another, suggesting that the segments are determined[33-35]. Clones produced a few hours later were restricted to either second leg or wing[33]. These results certainly indicate the appearance of a developmental segregation between ancestral cells for wing and leg in early development. Indeed, histological studies in another dipteran, *Dacus*, have shown that there is an initial group of cells of the mesothoracic segment that later become subdivided into dorsal (wing) and ventral (leg) imaginal disks[36].

Other evidence suggesting early determination comes from experiments in which homoeotic transformations are produced by chemical or heat treatment[37,38]; such changes are called phenocopies. For example, when early embryos are treated with ether as many as 30% of the flies show *bithorax* phenocopies[37,38]. They do not look exactly like flies mutant for *bithorax* because only part of the haltere is transformed into wing. These changes can be produced only at or around the blastoderm period[37,38], suggesting that the phenocopying agent interferes with some determinative process that takes place at that stage[38].

This evidence from clonal analysis and phenocopies does not tell us when imaginal disks are determined; in neither case has the total extent of the marked or transformed tissue been defined and it could include internal or larval organs. As individual disks appear as identifiable structures at late embryonic stages[39,40] they are probably determined by that time.

Determination and homoeotic genes

By the criteria usually used to define the state of determination, the homoeotic mutants change it. For example, antennal disks of *spineless-aristapedia* differentiate leg structures when transplanted into metamorphosing larvae[11]. In experiments where genetically marked cells from two different imaginal disks are dissociated, mixed together, and allowed to grow in an adult abdomen they usually sort out from one another[26,27]. While wild-type antennal and leg disk cells will segregate from each other, antennal disk cells from flies mutant for *spineless-aristapedia* will mix with wild-type leg disk cells[41]. In similar experiments, mutant haltere disks for *bithorax* differentiate wing structures in isolation and their cells will mix with wild-type wing cells[41,42]. Thus, by these criteria, the state of determination of the antennal disk is changed by *spineless-aristapedia* into that of the leg and the state of determination of the haltere is changed by *bithorax* into that of the wing. It follows that the normal function of homoeotic genes is necessary for the normal process of determination.

Since the disks become determined in early development, it is expected that the homoeotic genes will function early in development. There is some evidence that they do. In flies homozygous for extreme *bithorax* and *postbithorax* combinations (which transform the entire haltere into a complete wing, see cover), the number of cells of the mutant haltere disk is 2–4 times larger than the wild-type haltere disk in the first larval period and equal or similar to that of the wild-type wing disk[43]. This result indicates that the mutant phenotype is already expressed by the first larval period and therefore that the wild-type gene has already been required.

There are several experiments which indicate that there is, as well, a late requirement for the *bithorax* gene. When *bithorax* clones are produced in a wild-type haltere, the clones show mutant phenotype[4,43] (that is, they produce wing structures) even if they are induced before the end of the third larval period[43]. Only when the clones are produced in the late third stage larva do they fail to show mutant phenotype[43]. As we discuss below, the gene product may still be required after that time. A similar conclusion is indicated by some temperature sensitive alleles of *bithorax* where experiments have located the phenocritical phase in the third larval period[44]. Clearly, the activity of the *bithorax* gene is required until late in development of the third instar, but not thereafter. This ability of wild-type phenotype to persist in the absence of wild-type genes has been described for certain mutants affecting bristles, and called *perdurance*[45]. Although other explanations are possible, the simplest is that perdurance is the persistence in mutant cells of some wild-type function which is not diluted out in the last few cell divisions before the disk stops growing. This view is supported by the observation that late *bithorax* clones that show perdurance *in situ* (that is wild-type phenotype), differentiate mutant phenotype after additional growth in culture *in vivo* (Garcia-Bellido, unpublished). We have seen that there is evidence for *bithorax* function in the first stage larva, and that it is needed until metomorphosis. It seems likely, therefore, that the gene is active throughout most of larval development, and perhaps in the embryo as well (see ref. 43 for discussion).

Although the preceding discussion relies heavily on data on *bithorax* mutants, observations on other homoeotic mutants point to similar conclusions: there is evidence that the *engrailed* gene is required throughout most of the larval period for normal development of the cells of the posterior wing[6,46]. Its activity stops intermingling of anterior and posterior wing cells[46,47]. Indeed, as the anterior and posterior cells are first segregated at the blastoderm stage[34,35] but may lie adjacent to one another[48,35] the *engrailed* gene is probably required then. Mitotic recombination[6,47] and temperature shift experiments[47] show that wild-type function of the *engrailed* gene is required until the late third larval period. Mutant *spineless-aristapedia*, (ss^{a}), clones produced earlier than the mid-third larval period in a wild-type antenna show mutant phenotype (leg structures) but they retain wild-type phenotype when induced in the late third stage larva[49]. Also, the cold-sensitive allele ss^{a40a} has a phenocritical period in the third larval stage[50,51]. Both these observations are most simply explained as perdurance effects. Thus the *bithorax*, *engrailed* and *spineless-aristapedia* genes probably have a similar mode of action; they are responsible for the expression of specific states of determination by groups of cells. Specific disk cells require a continuous supply of the wild-type product of these homoeotic genes for the maintenance of their state of determination. If the activity of the homoeotic genes fails, the state of determination changes accordingly.

It now becomes easier to understand the instability of the state of determination in some hypomorphic (leaky) mutations like *Antennapedia* of *Rappaport*[10] or *Contrabithorax*[9]. In the first case the mutant antenna is a mixture of leg and antenna cells and in the second case the mutant wing is a mixture of wing and haltere cells. When clones homozygous for genetic markers are produced in the mutant appendages, it is found that even clones produced in the third instar can comprise leg and antenna in the case of *Antennapedia* and haltere and wing in the case of *Contrabithorax*. These experiments indicate that the decision whether to make one or another type of structure is not inherited through cell divisions but is made very late in development. Although these results have been interpreted as evidence for late determination in the mutant disks[10] they probably instead reflect the inherently unstable state of determination of the mutant cells that can change according to the amount of wild-type product present in each cell. Even during *in vivo* culture the homoeotic genes and their products are still required: *spineless-aristapedia* 40a has a phenocritical phase in the middle of the third larval period[50,51]. Mature antennal disks from the late third instar have passed the phenocritical period and have a strict state of determination which is independent of the temperature. However, if these same disks are cultured *in vivo*, the cells produced during the period of extra growth can become determined differently according to the temperature in which they grow[51].

Compartments and homoeotic genes

Clonal analysis of imaginal disks of *Drosophila* and the abdomen of the milkweed bug, *Oncopeltus*, have shown that there are certain boundary lines in the adult cuticle which are not crossed by clones after a given moment in development[52–55]. In *Drosophila* the introduction of the Minute technique[4,56] allows the production of marked clones that grow more rapidly than surrounding cells and reach a very large size. These clones can nearly fill certain regions

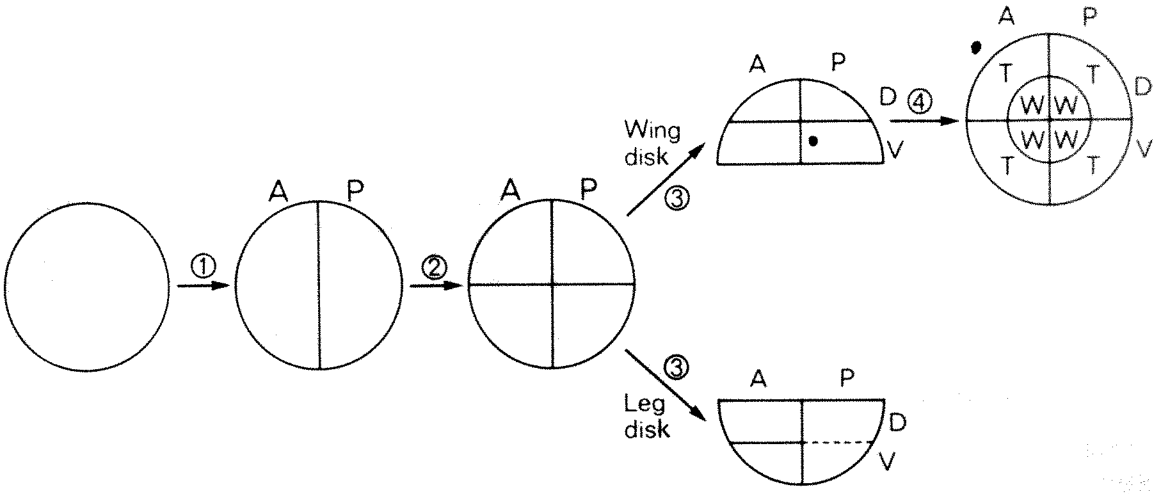

Fig. 2 Diagram of compartmentalisation of the mesothoracic cuticular structures. After each subdivision (represented by a straight line) no clone can include territories on both sides of that line. The first step (1) is the segregation of anterior (A) and posterior (P) polyclones, and this occurs, probably, at the blastoderm stage. The second (2) consists of the separation of the dorsal part of the segment (wing disk) and the ventral part (leg disk). This event takes place before 10 hours of development. These two disks later become physically separated. During the larval period the wing disk becomes subdivided (3) into wing dorsal (D) and wing ventral (V) and about the same time (4) into thorax (T) and wing blade (W). For the leg disk there is some evidence[34] of the subdivision of the anterior compartment into dorsal and ventral.

of the cuticle which are defined by precise boundaries in the adult. These regions are called compartments[4,5]. They are constructed by all the descendants of a particular group of founding cells (a polyclone[57]) which is set aside at an early stage of development. It has been shown that dividing cells cannot cross certain boundaries, and that some of these become effective before others[4,5], so that there is a successive subdivision of polyclones into daughter polyclones. In the cases studied so far the number of cells in the founding polyclones is small, being between 5–50 (refs 53, 54, 58–60, 61).

The assignment of a cell to a given polyclone is a stable developmental commitment; all the cell's progeny will be constrained to form structures within one specific compartment. In this way development can be visualised as a process in which homogeneous populations of cells are split into subpopulations with different developmental potential.

In *Drosophila* the formation of compartments has been studied for several imaginal disks[4,5,34] and has been reviewed recently[57,62]. Here we will discuss mainly the relationship between compartments, homoeotic genes and determination.

For the wing disk there are at least three subdivisions that produce eight major compartments. The wing is first subdivided into anterior and posterior regions and each subdivided further into dorsal, ventral, wing blade, and thoracic parts (Fig. 2). The first subdivision occurs so early that the mesothoracic disks (wing and second pair of legs) are subdivided into anterior and posterior polyclones before the wing-leg segregation occurs[34,35]. This is shown by clones produced about the blastoderm stage of embryonic development which include both anterior wing and anterior second leg structures or posterior wing and posterior leg. No clone has been found to transgress the antero-posterior compartment boundary in either leg or wing[34,35]. This observation shows that compartmentalisation begins before individual disks are formed. It is the whole mesothoracic polyclone (which forms the cuticle and perhaps other internal tissues as well) that becomes subdivided into anterior and posterior polyclones. The progeny cells of these anterior and posterior primordial polyclones will include all the anterior (leg and wing) and posterior (leg and wing) cuticular structures respectively. The probable sequence of compartmentalisation in the mesothorax is shown in Fig. 2.

An important feature of the antero–posterior compartment boundary is that it apparently defines the limit of function of certain homoeotic genes[4,5,46,47]. This is the case for the haltere-wing transformations produced by the *bithorax* and *postbithorax* mutants (both members of the *bithorax* pseudoallelic series[3]). The anterior wing structures produced by bx^3/bx^- (Fig. 1) in the haltere disk correspond to those defined in the ordinary wing by the antero-posterior compartment boundary, and the posterior wing structures produced by pbx/pbx^- correspond to the posterior ones defined in the normal wing by the same boundary. Thus *bithorax* and *postbithorax* have an effect on specific polyclones so that entire compartments are transformed. The same boundary is also recognised by the mutation *engrailed*. The activity of the wild-type function of *engrailed* is required by posterior wing disk cells: without it, these cells fail to develop both a normal posterior compartment and a normal antero–posterior compartment boundary[6,46,47].

Of other compartment boundaries described for the wing disk, the one separating proximal (thoracic) from distal (wing) structures seems to be the limit of the transformation produced by *wingless*. This mutation produces a duplication of the thoracic structures while the wing disappears[7]. As it affects the haltere disk in a homologous manner, the *wingless* gene may be involved in the development of the distal compartments of both the wing and haltere disks[8].

Determination begins when the original founder cells of the polyclone are chosen. Thereafter, during normal development, the cells of the polyclone can only form parts of a restricted region in the adult—the compartment. One would like to dissect the determinative process down to its operational elements, and some hints come from mutants like *engrailed* which produce duplicated compartments within one disk. It would seem that the subdivision of the polyclone into two sub-polyclones has proceeded normally, even though the homoeotic gene is defective. This suggests that, in normal development, there are at least two separable elements in each determinative step: (1) the definition of groups of cells and (2) the permanent activation of a homoeotic "selector gene"[62] in one or more groups. Garcia-Bellido[62] has coined the term selector gene to emphasise that there may well be more than one class of homoeotic mutation: homoeotic changes could result from mutations which affect the subdivision of polyclones, the genetic machinery leading to the activation of a particular homoeotic selector gene, or the selector gene itself.

The choice of particular groups of cells is probably geographic[57] and depends, for example, on polarity and positional information in the egg[63,64] or position in the disk. Homoeotic mutations in genes affecting these processes might be expected to have unusual properties, some might show a maternal effect. The gene products might only be required at an early stage of development, so that clones of cells mutant for these genes produced after that stage might show normal wild-type phenotype, *wingless* is, possibly, an example of such a gene[8].

The selector gene, as exemplified by *bithorax*, is a gene whose product is required continuously throughout development to 'select' a particular developmental pathway[62]. The role of the selector genes is of considerable interest; they seem to control the activities of many other genes which perform executive functions[62]. For example, the cell marker mutants are not restricted to particular compartments and their products are probably required by all cells that secrete cuticle. When the selector gene product is defective or missing all compartments where that selector gene is normally activated will be homoeotically transformed—which explains why single genetic lesions such as *engrailed* can produce homologous transformations in different disks.

Conclusions

At least in some cases, homoeotic mutations transform entire compartments. This indicates that the activity of these genes is restricted to specific polyclones. As we have seen the activity of homoeotic genes is necessary for the expression of certain states of determination. Thus the compartments seem to be the units of determination during normal development, the founding polyclone of a given compartment constituting the initial group of determined cells.

In this view, the final state of determination of the cells belonging to a compartment is the result of a series of events. Every determinative event is accompanied by the activation of a certain selector gene. Some of these events are common to different polyclones, but the combination of them, and therefore the combination of active and inactive selector genes, is unique to each compartment. Determination is a result of genetically controlled commitments made by *groups* of cells and passed on to all their progeny. The effect of each determinative step is conditioned by the previous ones, so that at each moment the cells react according to their developmental history.

Are there compartments and homoeotic genes in vertebrates?

It is not clear whether compartments occur in vertebrate development, and if they do, whether they are similar to those found in insects. The idea of lineage segregation is of course inherent to the germ layer theory, and it has been suggested before that vertebrate organs of one cell type may have their origin in a special group of cells[65]. Moreover, recent studies on early mouse development have shown there is a series of binary decisions, involving small numbers of cells (15-50, ref. 66), each apparently accompanied by changes in cell structure and possibly in cell adhesion, and each resulting in some restriction in developmental potential. For example, the first of these separates the prospective embryonic cells (inner cell mass) from the trophoblast. As in insects[57] these segregations appear to be made geographically[66].

In vertebrates determination has often been thought of as being to a particular cell type, such as muscle or nerve. Yet the picture coming from studies on *Drosophila* is one of commitment to a precise region; that is some kind of area property[57]. In vertebrates, detailed lineage studies could reveal whether a single organ or tissue is subdivided into compartments, but have not yet been undertaken. Other possible correlates of compartmentalisation might give further information. For example, some compartment boundaries in insects appear to be recognised by peripheral nerve axons[67], and a precisely placed line, which is respected by such axons, has been found separating the amphibian limb into two regions[68].

There is a lack of genetic evidence for compartments and homoeotic genes in mammals; this may not be significant since homoeotic mutants are more likely to be viable in insects, like *Drosophila*, where there is a complete metamorphosis and some adult structures (such as wings) are dispensable. These observations are certainly suggestive—even in the absence of detailed studies of cell lineage and the existence of frank homoeotic mutants.

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articles

Configuration of tobacco mosaic virus RNA during virus assembly

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When TMV reassembles, the uncoated RNA is folded back along the growing rod, probably down the central hole. This surprising configuration is essential for rapid elongation—presumably supplying RNA to its site of incorporation while keeping the bulk of the free RNA out of the way.

THE location of the nucleation region for the assembly of tobacco mosaic virus (TMV), from isolated coat protein and RNA, about 1,000 nucleotide residues from the nearer end of the RNA molecule (the 3′-hydroxyl terminus)[1,2] raises questions about the elongation process. Since the RNA is totally enclosed in the mature virus particles, elongation from the original site of nucleation must occur in both directions—towards the 3′- and 5′-hydroxyl termini of the RNA. This presents a paradox in view of the observation[3-7] that partially assembled rods of TMV have RNA tails only at one end, in agreement with the generally accepted view that assembly would be polar. We have shown that the two ends of the TMV rod can be distinguished in the electron microscope because one is convex and the other concave and, taking this together with stripping experiments, we have shown in which direction the RNA runs within the complete nucleoprotein helix[8]. We have now applied this technique to locate the free RNA in the growing nucleoprotein rods.

Preparation of rodlets

Partially assembled rodlets of the desired length range were obtained by allowing an appropriate amount of the protein, supplied as a disk preparation, containing about 80% of the 2-ring disk aggregate[9], to reassemble with the RNA[10] at 20 °C in sodium phosphate buffer, *p*H 7.0, ionic strength 0.1 for 10–15 min[9]. Rods about half the size of complete virus particles were obtained with a protein:RNA ratio of 10:1. Rodlets with free RNA tails at the 5′-hydroxyl end were prepared by partial alkaline stripping as before[8].

Both the rodlet preparations described were contaminated with free protein—either unreacted and possibly damaged in the partially assembled preparation or that which had been stripped off in the partially stripped preparation—and possibly some free RNA. These contaminants were much smaller than the rodlets and were removed before reassembly by centrifugation through sucrose gradients, without allowing the rodlets to pellet, as this alters their reassembly activities[9]. Gradients were prepared in Tris-chloride buffer, *p*H 8.0, ionic strength 0.1, by layering into tubes 2 ml 60% (w/v) surose, 2 ml 40·, 3 ml 20· and 2 ml 5· sucrose. Samples (3 ml) were layered on top and the gradients were centrifuged at 40,000 r.p.m. and 5 °C for 1 h in an MSE 6 × 14-ml titanium swingout rotor. Fractions were collected from the bottom of the tubes and the fractions from the turbid layer were pooled and dialysed against sodium phosphate, *p*H 7.0, ionic strength 0.1 containing 1 mM sodium azide.

The turbid suspensions had spectra characteristic of nucleoprotein, while the fractions from the tops of the gradients gave the spectrum of TMV protein, suggesting that little free RNA was present in the original preparations.

Location of RNA tails on growing rods

Although the uncoated RNA is not as clearly visible at the ends of partially assembled rodlets as on partially stripped rodlets, it can nevertheless be seen on many of the particles and the morphologically distinct ends of these particles can be distinguished. From Fig. 1 it seems that the RNA tails are at opposite ends of the two types of rodlet. This was confirmed by inspecting all the particles in

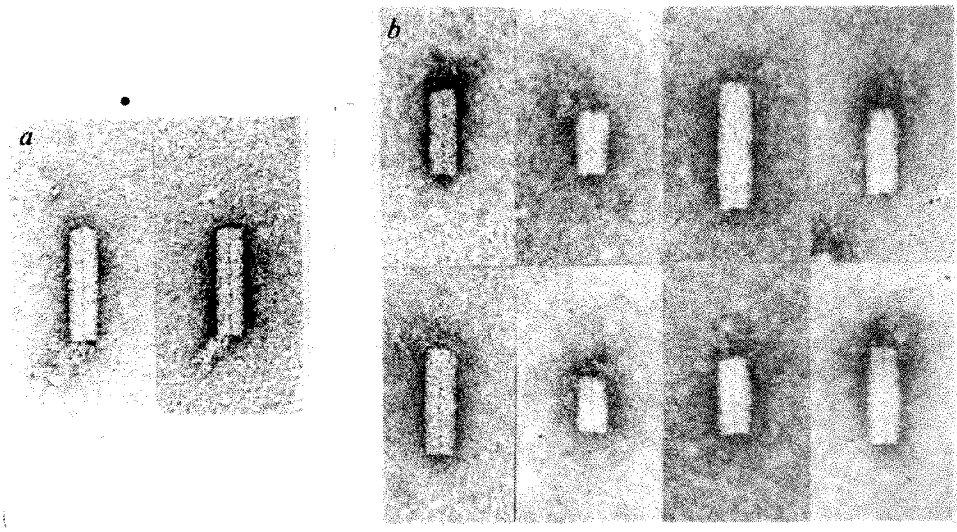

Fig. 1 Electron micrographs of TMV particles: *a*, partially stripped with alkali[8], and *b*, partially reassembled. The images are aligned with the rounded or convex ends at the top and the concave ends at the bottom. The 'puffs' of uncoated RNA can be seen at opposite ends in the two cases (approximately × 170,000). Specimens from the rodlet preparations were prepared by allowing the particles to adsorb on to carbon-coated grids from a drop of solution for about 30 s, followed by rinsing with distilled water and negative staining with 1% (w/v) uranyl acetate. The grids were examined and photographed in a Philips EM 300 electron microscope at a magnification of approximately 25,000.

Table 1 Numbers of particles for which the end with the exposed RNA is morphologically distinguishable

Type of rodlet	End showing RNA Convex	Concave	Both
Partially stripped[8]	1	62	6
Partially assembled	88	4	3

several fields of partially assembled rodlets and assigning the free RNA to the different ends whenever this could be done unambiguously. The counts are shown in Table 1, together with those for rodlets partially stripped by alkali using the same technique[8]. Clearly the RNA tails of the partially assembled rodlets occur at the convex ends of the particles in contrast to the situation found for partially stripped rodlets[8]. (Less than 8% of rodlets, whether single or double tailed, were identified with a tail at the concave end.)

Because TMV RNA is about 6,400 nucleotides long with the nucleation region about 1,000 nucleotides from the 3′-hydroxyl end[1,2] and hence more than 5,000 nucleotides from the 5′ end, the main direction of assembly must be from 3′ to 5′. In partially assembled rodlets the longer tail should therefore contain the 5′ end, which is the end released by alkali stripping[11]. As RNA tails are visible in partially assembled rodlets principally at the ends which contain the 3′ ends of the RNA in the final viruses, the RNA must be folded back on itself in the growing rods because it protrudes only from the end at which least elongation occurs (Fig. 2).

Rates of elongation

We suggest that the looping back of the 5′ end of the RNA in the growing rods, probably down the central hole, is important for the mechanism of elongation of the rods in the dominant direction, from 3′ to 5′. In this case we expected that the rates of elongation of partially assembled rods and of partially stripped rodlets might be different because, although both would be elongating in the 3′ to 5′ direction, the long RNA tail would be tucked down inside the former but protruding from the stripped end of the latter.

This prediction was investigated using rodlets which had been purified and checked in the electron microscope to ensure that most still had RNA tails. Protein for elongation was supplied both as a disk preparation and as A-protein. The results of one such experiment, in which the partially assembled rodlets and two preparations of partially stripped rodlets were purified in parallel, including the final dialysis in the same flask, to eliminate any possible effect of preparative history, are shown in Table 2. Although the large molecular sizes and unknown molecular weights of the preparations make it unsafe to try to calculate the absolute rates of elongation from these rates of increase in turbidity, or even to make a close comparison of the rates between different preparations, the partially assembled rodlets seemed to elongate more than an order of magnitude faster than did the partially stripped rodlets. This conclusion remains, even allowing for the greater concentration of the former.

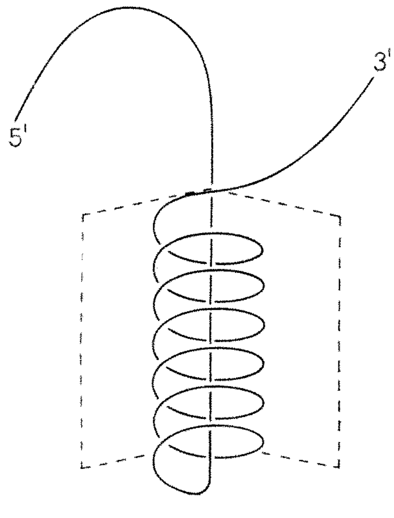

Fig. 2 Schematic picture of the suggested arrangement for the RNA in a growing rod. The dominant direction of assembly (that is, from 3′ to 5′) is downwards—at the end opposite the two RNA tails. The placing of the longer, 5′ tail up the central hole is arbitrary and, while we think this is the most likely configuration, there is no direct proof as to how it reaches the opposite end of the rod.

A compatible result was obtained when the lengths of partially stripped rodlets were compared by electron microscopy before and after incubation with a disk preparation at 1.0 mg ml^{-1} for 30 min. While partially assembled rodlets would, in these conditions, be expected to elongate by 150–200 nm (refs 9,12), the partially stripped rodlets only increased by about 10 nm. Although a ^{2} analysis on the histograms of lengths of the rodlets showed that even without genuine elongation, such and apparent change could have arisen from sampling with a probability of just under 0.1, we think that elongation had taken place, as it has a 90· probability of doing. Moreover, rods partially stripped from the same end with SDS[8] will reassemble over several hours to give nuclease resistant infectivity[13], in agreement with the idea that some slow elongation can probably occur even with the RNA tail free at the abnormal end of the growing rod.

Preliminary location of both tails

The observations of the RNA tails described above were made with negatively stained specimens, so that high resolution electron microscopy distinguished the ends of the rodlets, but in these conditions the RNA tails only appear as 'puffs' and it is not possible to distinguish between one or two tails at the same end of a rod, but rather the major tail (the 5′ end) dominates the image. We have occasionally seen partially assembled particles apparently with two RNA tails coming from the same end, by electron microscopy of specimens shadowed after spraying onto carbon coated grids with only bovine serum albumin as a spreading agent. We do not regard these observations as very significant because most of the particles showed only a single tail, and some free RNA was visible in the background. To obtain better spreading and separation of the tails it is probably necessary to add some denaturing agent. Preliminary experiments, however (unpublished results of B. Jacrot and D.Z.), showed that partially assembled rods are very unstable when RNA is denatured for spreading, although partially stripped rodlets could be visualised. This again shows the difference between these types of rodlet, but emphasises the need for extreme caution in interpreting such data because preparation may significantly alter the specimen.

An alternative approach to visualise and distinguish the ends of the RNA is to label them with a bulky molecule. We have done some preliminary experiments using ferritin, activated for reaction with periodate-oxidised RNA[14] by coupling to carbohydrazide with ethyl dimethylaminopropyl-carbodi-imide[15,16], to label both termini. One problem is that the tendency to form ferritin aggregates could cause confusion. Although we have never seen a particle with ferritin molecules at both ends, we have seen several partially assembled rodlets on which two ferritin molecules appear, in the electron microscope, to be attached to the same RNA 'puff' and yet are too far apart to be attached directly to each

Table 2 Rates of reassembly of partial TMV rodlets

Rodlet preparation	Rate of turbidity increase (D_{310} per min) Disk preparation	A-protein
Partially stripped I	0.00125	0.0006
Partially stripped II	0.00115	0.00065
Partially assembled	0.0316	0.0221

Rodlets were prepared either by partial alkali stripping or by partial reassembly. Elongation in sdium phosphate, *p*H 7.0, ionic strength 0.1 at 20° C, was followed by the increase in turbidity at 310 nm (ref. 9), with either a disk preparation or A-protein added to 1 mg ml^{-1} final concentration. Final rodlet concentrations were 0.20 and 0.195 D_{260} per ml for the partially stripped rodlets I and II and 0.42 D_{260} ml^{-1} for the partially assembled rodlets (corresponding to approximately 0.6, 0.5 and 1.2 mg ml^{-1}, respectively).

other. We believe that this indicates that the two RNA termini are indeed at the same end of the assembling rodlet and we are continuing experiments to confirm this.

Implications for assembly

The structure of the protein disk is known to a resolution of 5Å (ref 17) and consists of two rings, each of 17 protein subunits, stacked on top of each other. While these make a firm contact at high radius, they are separated vertically at the RNA-binding radius, like a pair of 'jaws', and the protein has considerable flexibility inside this radius. These features suggested that the RNA might well be intercalated between the two rings of the disk, during both nucleation and elongation, from the central hole of the disk and in this way the two-layer disk could be incorporated into the single helix.

Although there is no firm evidence of whether the doubling back of the longer RNA tail occurs inside or outside the growing nucleoprotein helix, we suggest that it is probably inside with the RNA strand running freely down the central hole (Fig. 2). This configuration in the growing rod would follow smoothly from a mechanism of nucleation by the insertion of a hairpin loop of RNA into the centre of the first disk[18]. Such a geometry will allow access of the relevant region of the RNA to its binding sites on both rings of an incoming protein disk and yet hold the bulk of this RNA strand well out of the way of the region of activity, greatly facilitating the process of rod elongation.

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A model for superlight velocities of extragalactic radio sources

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An explanation is proposed for apparent superlight velocities of compact radio sources. In our model high velocities are a consequence of the interaction of the radio sources with the ambient gas in a galactic nucleus or quasar. This model predicts that the pair of sources is not centred on the nucleus and that the individual components should be smaller and more separated when observed at higher frequency.

APPARENT faster-than-light velocities of separation have been measured with VLBI for compact double radio sources in 3C120, 3C273, 3C279, and, most recently, in 3C345 (refs. 1-8). Furthermore, in a number of low-frequency sources the time-scale for luminosity changes corresponds to apparent expansion velocities in excess of the speed of light[9].

Many explanations have been offered for these perplexing observational results; however, the models proposed so far either contain implausible assumptions or predict phenomena which have not been observed, such as double sources in which the components move toward each other or in which the components have very dissimilar luminosities[2-7,10-13].

From observations of extended extragalactic radio objects it is known that mechanisms exist for ejecting energy sources in well defined directions from the centres of galaxies and quasars[14,15]. We show here how the motion of one of these energy sources through the ambient gas in the centre of a quasar or galactic nucleus can produce a pair of bright radio emission regions which separate at superlight velocities or can produce rapid luminosity changes. The model we propose thus provides suggestive connections among the observed properties of quasars and galactic nuclei, and extended and compact radio sources. Our model is an extension of the expanding radio source models of Shklovskii[16,17] and van der Laan[18] which have been remarkably successful in describing the variable emission from 3C120 (ref. 19). In their models a stationary source creates a spherical cloud of relativistic electrons and magnetic field which expands adiabatically. While the source is highly compact, it is optically thick due to synchrotron self-absorption. As the cloud expands, the flux at a given frequency increases until the cloud reaches some critical radius, at which it becomes optically thin at this frequency; thereafter the flux decreases.

Our model differs from earlier models in that we allow the source of particles and/or field to move through an ambient medium of varying density. It is easy to see that apparent superlight velocities can be obtained by this generalisation. Consider the evolutionary sequence in which a source moves out from the centre of a galaxy or quasar with a velocity $v_s < c$. As the energy source moves through the ambient gas it creates a cavity in its wake which is filled with particles and magnetic field. In the regions where the cavity has expanded to a critical radius R_c, corresponding to unit optical depth, the radio surface brightness is at a maximum. This bright region—the apparent position of the radio object—lags behind the actual energy source by a distance $v_s\tau_{exp}$ where τ_{exp} is the time for the cavity to expand to R_c. If the cavity is confined by the ram pressure of an external medium of density ρ, then the expansion velocity is proportional to $\rho^{-1/2}$ and $\tau_{exp} \propto \rho^{1/2}$ (ref. 20). Because the density of the ambient gas decreases away from the centre of the galaxy or quasar, the lag distance decreases as the source moves outward. The apparent position of the radio-emitting region tends to catch up to the energy source. The apparent velocity is thus greater than the actual physical velocity of the energy source. Because this apparent velocity is merely due to a phase-delay effect—the delay of the brightening of the radio object is greater when it is embedded in higher density gas—arbitrarily large velocities could, in principle, be obtained.

A single moving energy source can actually produce two bright emission regions which separate at superlight velocities. If

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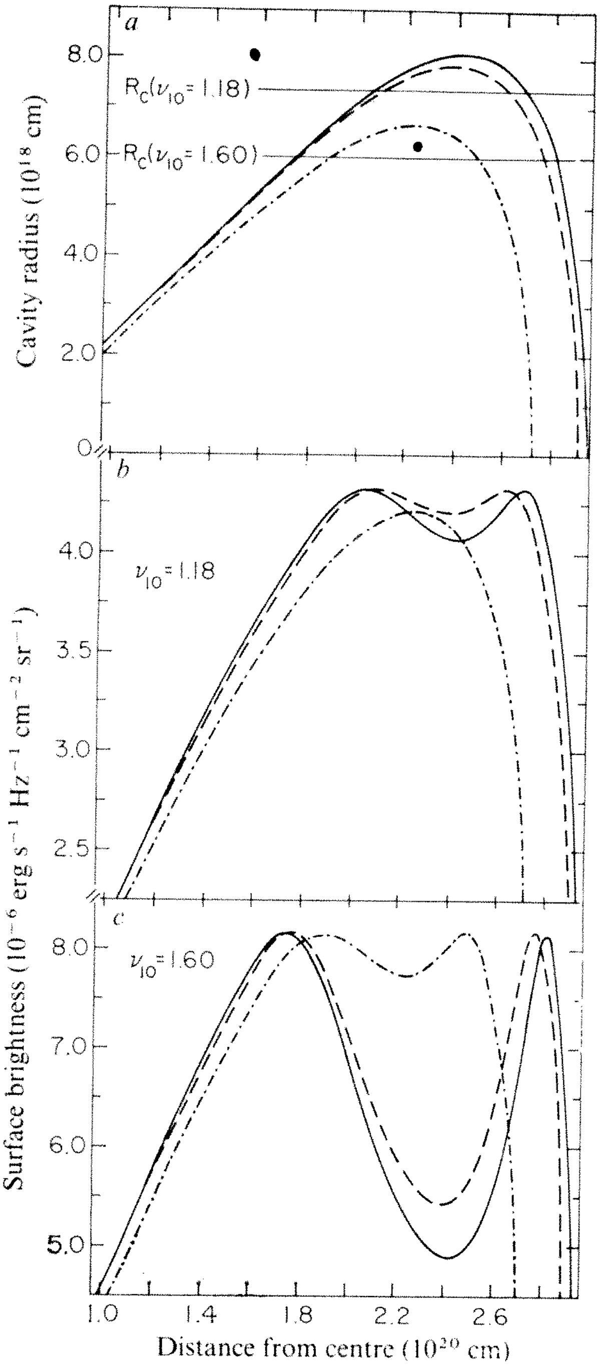

Fig. 1 The evolution of a compact radio object is illustrated at three epochs. *a* The evolution of the cavity boundary. The critical radii for two frequencies are indicated here. *b*, *c* The evolution of the surface brightness along the symmetry axis of the cavity for each of these frequencies. –·–·–, $t = 1.830 \times 10^{10}$ s; – – –, $t = 1.955 \times 10^{10}$ s; ———, $t = 1.980 \times 10^{10}$ s.

the ambient gas is sufficiently concentrated toward the centre of the galaxy or quasar, the cavity expands very slowly in these dense regions and has its maximum diameter somewhere between the centre of the quasar or galaxy and the location of the energy source. This point is illustrated in Fig. 1. Figure 1*a* shows an example of an evolutionary sequence for the cavity radius; the critical radii for two frequencies are indicated. Figure 1*b* and *c* show the corresponding evolution of the brightness distribution.

Consider the cavity to be divided into disk-like segments of fixed thickness along the direction of motion. As each segment expands, the brightness at a given frequency increases until the segment reaches a critical radius; the brightness then decreases as the segment expands further. As long as the radius of the cavity is everywhere less than the critical radius, the brightness distribution exhibits a single maximum which coincides with the position of the maximum cavity radius (cf., brightness distribution for $\nu_{10} = 1.18$ at $t = 1.83 \times 10^{10}$; where ν_{10} is the frequency, units 10^{10} Hz). With further expansion, some portions of the cavity exceed the critical radius. The brightness distribution then becomes bimodal and the regions of greatest brightness occur where the cavity radius is equal to the critical radius.

When the maximum radius of the cavity is only slightly greater than the critical radius, the two brightness maxima separate with arbitrarily large velocity, but the brightness distribution has a very shallow minimum. During the subsequent evolution the apparent separation velocity decreases and the bimodal structure of the brightness distribution becomes more pronounced.

As the energy source moves into regions of lower density, the separation between the leading bright region and the source decreases due to the decreasing τ_{exp}. The leading bright region therefore moves forward faster than the energy source. The trailing object moves towards the centre of the galaxy or quasar with a smaller, but comparable velocity so that the two bright regions separate with an apparent velocity which is significantly greater than the velocity of the energy source and which can easily be superluminal.

This mechanism for producing rapidly separating pairs of bright regions has a well-defined signature which may be deduced from the frequency dependence of the critical radius and the geometrical nature of the model. Since synchrotron-self absorption decreases with frequency[21,22], the critical radius is larger at lower frequencies as indicated in Fig. 1*a*. As can be seen from the figure, a pair of bright regions observed simultaneously at two different frequencies should have the following characteristics: at the lower frequency which corresponds to a larger R_c (1) the two sources are closer together, (2) the brightness minimum between the two sources is less well defined and (3) the individual component radii which are roughly proportional to R_c are larger.

To make more quantitative estimates of the physical structure and brightness distribution of a radio source, one must formulate an explicit and hence more elaborate model. Any such model requires a number of simplifying assumptions about the gas distribution in the centres of galaxies and quasars, about the nature of the energy source and about the mechanisms which operate within the cavity. A model of this sort, though at best heuristic, can serve to illustrate the general interrelationships among the properties of compact objects, galaxies and quasars and extended radio sources.

The model

The gas density in the galaxy or quasar is assumed to be constant near the centre and to decrease at some power law at large distances, z, from the centre according to the relation

$$\rho(z) = \rho_0(1 + z/z_0)^{-n} \tag{1}$$

where ρ_0 is the central density and z_0 is a core radius. The energy source moves away from the centre of the density distribution with a constant velocity v_s and deposits energy at a uniform rate in the form of relativistic particles and field.

It is assumed that each segment of the cavity evolves independently in two dimensions; that is, energy flows along the length of the cavity are assumed to be small compared with the work done by the cavity in expanding in the radial direction. This approximation is reasonable for the model presented here. For the highly elongated cavities, the pressure gradients along the cavity are small in the regions of interest; thus the time scale for energy flow along the cavity should be long compared with the typical radial expansion time.

The rate of expansion of the radius of the cavity at a given distance, z, is determined by the balance of the internal pressure, P_i, and the ram pressure of the external medium, $\rho\dot{r}^2$

$$\dot{r}(z,t) = (P_i/\rho(z))^{1/2} \text{ for } t > z/v_s \tag{2}$$

The internal pressure derives from the magnetic field, assumed to be chaotically oriented, and from the relativistic electrons and heavy particles. The energy densities of the magnetic field and of the relativistic electrons are, respectively, $U_M = B^2/8\pi$ and $U_e = \delta U_M$. The internal pressure is defined as κU_M, where the parameters δ and κ are constants of the energy source. For each slice of the cavity, the evolution of the magnetic field is prescribed by the relations

$$B = B_0 \quad \text{for } 0 < r \leq R_0 \tag{3a}$$

and

$$B = B_0 (r/R_0)^{-4/3} \quad r > R_0 \tag{3b}$$

The first phase (equation (3a)) is the input of energy at a rate

$$W = 3\kappa B_0^2 R_0^2 v_s/4 \tag{4}$$

This rate corresponds to filling the cavity of radius R_0 with particles and magnetic field and to accelerating the ambient gas of the cavity to the velocity given by equation (2). During the second phase (equation (3b)), the cavity expands adiabatically in two dimensions.

To complete the description of the model the energy distribution of the relativistic electrons must be specified. Initially, the distribution is taken to be a power law in energy at high energies

$$N(E)\,dE \propto E^{-m}\,dE \quad E > E_c \tag{5}$$

where $m > 2$. A specific choice of m, E_c and δ defines the proportionality constant in equation (5). We assume that the evolution of the spectrum is entirely determined by adiabatic changes which preserve the power law spectrum and require that $E \propto B^{1/2}$. This implies that other energy loss processes are either inconsequential or are counterbalanced by ongoing electron acceleration. In the case where the source velocity is perpendicular to the line of sight, the intensity of the radiation segment of the cavity of length dz is approximated by

$$I dz = 2r dz \frac{\eta}{\mu} (1 - \exp(-\tfrac{1}{2}\pi r \mu)) \tag{6}$$

where η and μ are the vacuum synchrotron emissivity and opacity, respectively. Expressions for η and μ are taken from refs 21 and 22. The surface brightness is approximately $I/2r$.

The parameters chosen for the model illustrated in Fig. 1 are $v_s = 1.5 \times 10^{10}$ cm s^{-1}, $\rho_0 = 1.72 \times 10^{-8}$ g cm^{-3}, $z_0 = 2.2 \times 10^{19}$ cm, $n = 10$, $B_0 = 3.8$ G, $R_0 = 1.4 \times 10^{18}$ cm, $\delta = 0.01$, $\kappa = 2$, $m = 3$. The parameters are chosen so that the expansion velocity is non-relativistic during the period of interest. The two frequencies $\nu_{10} = 1.18$ and $\nu_{10} = 1.6$ correspond to observing wavelengths of 3.8 and 2.8 cm for a source with a redshift $z = 0.5$.

At the first epoch illustrated in Fig. 1, the source is about 600 yr old, but the double structure, the large apparent separation velocity and high luminosity are just beginning to emerge. The second and third epochs are separated by 8.3 yr, characteristic of an observing interval. Between the second and third epochs, the peaks of the brightness distribution at both frequencies separate with average velocities greater than c. The low frequency brightness peaks are 40% closer together and separate at a velocity about 25% higher than the high frequency brightness peaks.

For the parameters of our model B_c, the field in the region of the cavity where the radius equals R_c, is ~ 0.1 G.

An electron which radiates at about 10^{10} Hz in this field has a radiative lifetime of the order of a few years, which is much shorter than the age of the source. As presently formulated, the model thus requires that some mechanism continually replenish the higher energy electrons. This requirement is not a serious problem for the model. The necessity of some sort of *in situ* acceleration is a feature of many radio source models[15], and the model parameters can be chosen to make the energy in the magnetic field sufficient to compensate for radiation losses for several thousand years.

For $B_c \sim 0.1$ G, the rate of energy input to the cavity (equation (4)) can be estimated as

$$W \sim 2 \times 10^{46}\,\kappa\,(R_c/R_0)^{2/3}\,(v_s/c) \text{ erg s}^{-1} \tag{7}$$

(see equations (3b) and (4)). The total energy which is supplied to a cavity of length Z is WZ/v_s. For a cavity 100 pc long, this energy is of order 10^{57} erg.

The ambient gas density at the point, z_c, where the double source structure first appears can be derived from equation (2). Integration of this equation from time z/v_s to t yields an expression for the cavity radius. Solving for the gas density at the location of the maximum cavity radius gives

$$\rho(z_c) \sim 10^{-22}\,\kappa \left(\frac{B_c H_\rho c}{R_c v_s}\right)^2 \text{ g cm}^{-3}. \tag{8}$$

Here $H_\rho \equiv \rho/(d\rho/dz)$ is the density scale height at z_c. There is approximately a factor of 2 uncertainty in equation (8) due to the choice of R_0/R_c.

In order to achieve large apparent velocities the density scale height has to be of the same order as the separation of the bright regions; H_ρ/R_c is less than about 10. For this sort of density gradient equation (8) yields densities of 10^{-21} to 10^{-18} g cm^{-3}. The central density of the model shown in Fig. 1 is actually much greater than the above estimates; however, this high density is an artefact of the simple density distribution which was used (equation (1)) and does not provide an important physical constraint on the model.

The skewing of the cavity apparent in Fig. 1*a* is directly related to the steep density gradient. The lower gas density near the energy source allows a more rapid radial expansion causing distension towards the source. The positions of the leading bright regions at different frequencies, as determined by the intersections of the cavity boundary with the critical radii in Fig. 1*a*, are thus fairly close together. For steeper density gradients, the positions of these bright regions would be even less frequency dependent.

Discussion

We have shown that a source of relativistic electrons and magnetic field which moves out through the gas in the centre of a galaxy or quasar can produce two bright emission regions which separate at superlight velocities. The energy source must travel at a few tenths the speed of light and must deposit about 10^{57} erg over several tens of pc. Our model is not dependent on the specific nature of the energy source or the detailed mechanism for providing directionality. The source is perhaps a rapidly moving condensed object[23,24] or simply a region where a beam of plasma interacts with the ambient gas[25-27]. The densities and scale heights which are required to yield rapidly separating double radio sources are consistent with the estimates of the properties of galactic nuclei and quasars which are deduced from optical studies[28].

The basic phenomenon described here has several distinctive characteristics which are essentially model independent: the bright regions should be smaller, more separated and have deeper minima between them when observed at higher frequencies. Furthermore, the pair of bright regions should not be symmetric with respect to the centre of the optical brightness distribution of the galaxy or quasar.

Within the context of the specific model formulated here, the structure of the bright regions is sensitive to the gas density scale-height; the structure is less frequency dependent when the density gradient is steeper. In active galactic nuclei or quasars the required steep density gradients would develop as a result of violent events. Some of the assumptions which were made to develop the simple model shown in Fig. 1 should be relaxed to correspond more closely to the actual physical reality. One might, for example, expect to find irregularities in the density distribution, in the rate at which the source supplies energy, or in the source velocity. These may lead to fluctuations in the brightness of the components and in variations in the apparent velocity of separation. In addition, the source velocity need not be perpendicular to the line of sight. Interesting relativistic effects occur when a bright region moves at a high or superluminal velocity at small angles with respect to the line of sight[11,12,29]. The apparent separation velocities of the bright components can be enhanced, and a single bright region moving toward the the observer may appear as two rapidly separating components.

The expansion velocity of the source itself may be relativistic. Existing models which employ relativistic expansion to explain rapid variability in radio sources would imply extremely high velocities in some exceptional cases[9]. This rapid variability is more easily explained in terms of a highly extended cavity which expands relativistically: because the cavity can be elongated by a factor of 20 or more when maximum brightness is attained, the surface over which coherent expansion occurs is greatly increased. It should be noted that rapid variations observed at one frequency may indicate that at higher frequencies the source may appear as two rapidly separating components.

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letters to nature

A simple physical model for X-ray burst sources

THE observational properties of X-ray burst sources have recently been reviewed by Grindlay[1] and Lewin[2]. These sources are concentrated in the direction of the galactic bulge and near the galactic plane. If they are at a mean distance of ~ 10 kpc and the burst radiation is emitted isotropically, then the time-averaged luminosities in bursts are typically ~ 10^{34}–10^{36} erg s^{-1}. Several of these sources may also have relatively steady X-ray luminosities[2]. Because of the limited sky coverage of available X-ray astronomy satellites, it seems likely that there are many times more burst sources in the galaxy than have heretofore been discovered.

There are ~ 150 known "steady" galactic X-ray sources[3], which are also concentrated toward the galactic bulge and in the galactic plane and have luminosities of up to ~ 2×10^{38} erg s^{-1}. The most widely accepted model for many of these sources consists of a compact object that is accreting matter from a close binary stellar companion[4]. For many of the galactic-plane sources with identified optical counterparts, the companion stars are OB supergiants, and there is some evidence that these stars nearly fill their critical potential lobes (see, for example, refs 5 and 6). The wind emitted by the companion star may well be enhanced by its proximity to its critical lobe[6,7]. Although the evolutionary time scale of an OB star fromthe main sequence to the supergiant stage is about 10^6–10^7 yr, it has been argued on statistical and theoretical grounds that a binary system containing such a star and a compact object becomes a bright X-ray source for only ~ 10^4–10^5 yr preceding the filling of the critical lobe of the OB star (ref. 7; ref. 8 and references therein).

The above information suggests a simple physical model for an X-ray burst source in the galactic disk, comprised of an unevolved OB star with a relatively weak stellar wind and a compact object in a close binary system. For some (as yet undetermined) reason, the stellar wind from the OB star is unable to accrete steadily on to the compact object unless the accretion rate, and hence the average X-ray luminosity, exceeds some critical value. Thus, when the stellar wind is sufficiently weak, the compact object accretes irregularly, leading to X-ray bursts. If this picture is correct, then many present burst sources will evolve into bright "steady" X-ray sources when the associated OB stars evolve and approach their critical lobes.

Accretion by a compact object from the weak stellar wind of a companion is also a tenable model for the burst sources associated with the galactic bulge. However, the stellar population of the galactic bulge is probably very different from the disk population, and the evolutionary histories and other detailed properties of the bulge burst sources are thus likely to be different from those of the disk burst sources. (The apparent association of three burst sources with globular clusters (refs 1 and 2 and references therein) strongly supports the identification of a subset of these sources with Population II objects.)

A magnetised neutron star is the most plausible candidate for the compact object in an X-ray burst system[9]. Theoretical studies have indicated that the character of spherical accretion on to a magnetised non-rotating neutron star changes[10], and that steady accretion might in fact not occur (F. K. Lamb, A. C. Fabian, J. E. Pringle, and D. Q. Lamb, submitted to *Astrophys. J.*), if the time-averaged X-ray luminosity $\lesssim 10^{36}$ erg s^{-1}. The rotation period of the neutron star may also play a critical role in determining the steadiness of the flow[9].

For rotating neutron stars with sufficiently low luminosities or sufficiently short rotation periods, the corotation velocity in the vicinity of the Alfvén surface will be larger than the circular Keplerian orbital velocity. This results in an effective centrifugal barrier[9-11] that should inhibit the steady accretion of matter and provide a mechanism for the storage of matter above the neutron-star surface (ref. 9; ref. 12 and references therein). For a $\sim 1\ M_\odot$ neutron star of radius $\sim 10^6$ cm, corotation at the Alfvén surface is determined by the following approximate relation (see refs 11, 13) between the rotation period, P, the time-averaged luminosity, L, and the neutron-star magnetic moment, μ

$$P \sim 0.2\left(\frac{L}{10^{37}\ \mathrm{erg\ s^{-1}}}\right)^{-3/7}\left(\frac{\mu}{10^{30}\ \mathrm{G\ cm^{3}}}\right)^{6/7}\ \mathrm{s} \qquad (1)$$

For more rapid rotation, matter is likely to accumulate and compress the Alfvén surface until accretion on to the neutron-star surface can proceed. Under these evidently unstable conditions, bursting behaviour may result.

The observed values of X-ray luminosity and rotation period for the nine known binary X-ray pulsars are shown in Fig. 1. It is likely that these objects are all rotating magnetised neutron stars undergoing accretion of mass from binary stellar companions (ref. 14 and references therein). The relation $P \propto L^{-3/7}$ is superimposed on the figure and normalised so as to pass through the parameter values of Hercules X-1, which seems to be in a near balance between spinup and spindown (ref. 12 and references therein). Most of the X-ray pulsars lie substantially above this line; the 'missing' neutron stars that lie below this line may be X-ray burst sources.

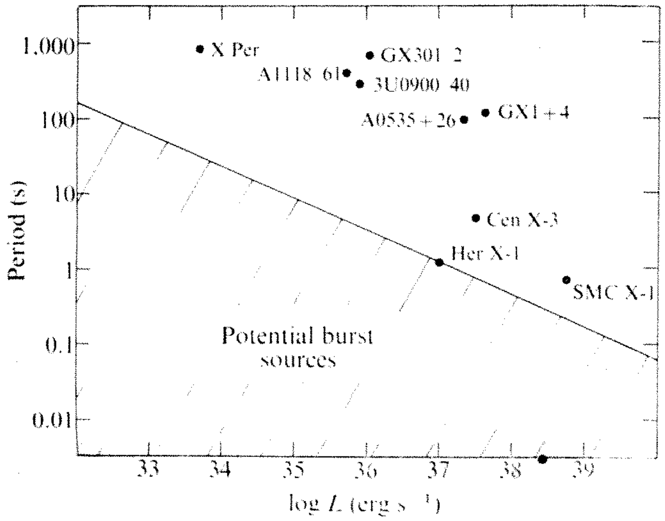

Fig. 1 Observed values of typical X-ray luminosity, L, and pulse period, P, for nine X-ray pulsars (see ref. 19). (For the transient sources A1118–61 and A0535+26, the maximum observed luminosity is indicated. The indicated pulse periods and luminosities are taken from ref. 20 and references cited therein. The straight line with logarithmic slope of −3/7 represents the relation between X-ray luminosity and pulse period such that matter in a circular Keplerian orbit will corotate with the neutron star at the Alfvén surface[11,13,19]. The line is normalised so as to pass through the parameter values of Hercules X-1 (see text). For neutron stars which lie below this line, matter that reaches the Alfvén surface cannot corotate because its rotational velocity would then exceed the circular Keplerian orbital velocity, and such matter may therefore be unable to accrete steadily across the surface[9,11].

We note that X-ray bursts have been observed from the direction of the bright 'steady' sources Serpens X-1 (3U1837+04), A1905+00, MX1728−33, and 3U1820−30, to within positional accuracies of $\sim 0.1°$ (ref. 2 and references therein). Other burst sources may also have substantial 'steady' luminosities (refs 1, 2, and references therein). If the observational identification of bursts with bright 'steady' sources is confirmed, then the presence of bursting behaviour in a binary stellar system containing a compact object must depend upon additional parameters besides the time-averaged luminosity. For example, if the burst sources are rotating neutron stars, then the neutron-star rotation period and magnetic moment presumably also have a role in determining the existence of bursting behaviour[9] (see equation (1)).

If our suggested identification of burst sources with binary systems containing compact objects is correct, then several predictions are possible. First, as more burst sources are discovered, the spatial distribution of the burst sources should follow the distribution of the 'steady' galactic sources (although the ratio of galactic-bulge objects to disk objects may be different for the burst sources than for the 'steady' sources). Second, the ratio of the total number of disk burst sources to "steady" disk sources should be comparable to the ratio of the main-sequence lifetime of an OB star to its lifetime in a state of enhanced stellar wind as it approaches its critical potential lobe (that is, $\sim 10^1$–10^3). Third, for some sources with slowly varying 'steady' X-ray luminosities, one should see transitions between bursting behaviour when the time-averaged luminosity is low and no bursts when the luminosity is high. This phenomenon has already been observed in the source 3U1820–30 (ref. 15). Other examples of this type of effect might be the onset of bursting behaviour during the extended inactive states of transient sources and Hercules X-1-type systems. Fourth, the existence of very large bursts from relatively nearby binary systems ($\lesssim 1$ kpc) is predicted. For some of these sources, it may be relatively easy to identify the optical companion star. It has previously been suggested[16,17] that the high-energy tail of nearby X-ray bursts could account for the observed γ-ray burst events[18]. However, we note that the repetitive nature of the bursts from many of the X-ray burst sources is apparently not characteristic of the γ-ray sources.

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Detection of change in rotation measure of the Vela pulsar

OBSERVATIONS made over the past six years show that the rotation measure of the Vela pulsar has increased by 14%, while over the same period the dispersion measure has shown a marginally significant decrease of about 0.2%. These results imply that a region containing a relatively strong magnetic field has moved across the path to the pulsar. This region of strong field is most probably associated with filamentary structure in the Vela supernova remnant.

Using a polarimeter giving simultaneous measurements of all four Stokes parameters[1] we obtained observations of PSR 0833−45 at well separated frequencies on three occasions, December 1971, July 1975 and June 1976, as shown in Table 1. Except for the 338 MHz measurements, data were obtained at several frequencies within the bands given in Table 1, the measurements being simultaneous at either two or three frequencies within a band. Observations in different bands were separated by a few days for the 1971 and 1976 observations and by 3 weeks for the 1975 observations. These observations permitted accurate determinations of the rotation measure along the path to the source and of the position angle of the emitted radiation extrapolated to infinite frequency, the intrinsic position angle. The results of these measurements are given in the table and are plotted in Fig. 1 together with data from Komesaroff *et al.*[2,3], who observed the source in May 1970 at a number of frequencies simultaneously using a single linearly polarised feed at each frequency. The rotation measure of the Vela pulsar was determined at earlier epochs by Radhakrishnan and Cooke[4] and Ekers *et al.*[5], but their data are less precise than the more recent measurements and so are not included in Table 1. All rotation measures have been corrected for the effects of Faraday rotation in the Earth's ionosphere using concurrent measurements of the critical reflection frequency f_0F2. The correction was in the range of 0.7 – 1.3 rad m^{-2} for these observations and is estimated to be uncertain by less than 10%.

It is clear from Fig. 1 that there has been a significant change in the rotation measure along the path to the source during the past 6 yr. The fact that there has been no significant change in the intrinsic position angle confirms that the observed changes result from variations in the rotation measure rather than in the emitted position angles. It also provides an *a posteriori* justification for the assumption of a ν^{-2} position angle dependence between the different frequency bands.

For the 1975 and 1976 observations, dispersion measures were also computed from the 631–649 MHz data; the results are shown in Table 1 and Fig. 1. There is some indication that the dispersion measure has decreased between 1970 and 1976.

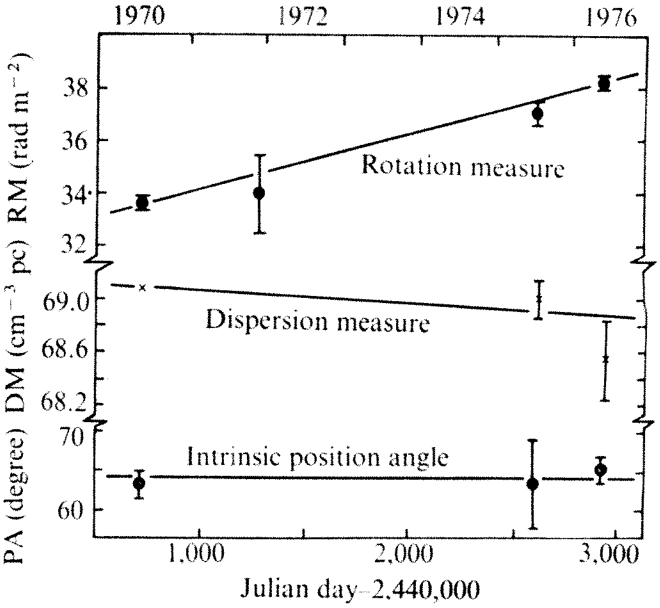

Fig. 1 Rotation measure, dispersion measure and intrinsic position angle of PSR 0833−45 at different epochs. For the rotation and dispersion measures the lines represent least-squares fits to the data, whereas for the intrinsic position angle the line has zero slope.

For the rotation and dispersion measure data, the straight lines in Fig. 1 represent weighted least-squares fits to the observations and have slopes of 0.77±0.04 rad m^{-2} yr^{-1} and 0.033±0.030 cm^{-3} pc yr^{-1} respectively (1σ errors). If the dispersion measure change is real and associated with the rotation measure change, then the implication is that a region of excess electron density has moved out of the path to the pulsar during the past 6 yr. Since the rotation measure increased during this time, the mean line-of-sight component of the magnetic field in this region must have been directed toward the pulsar giving a negative rotation measure contribution. This is in the opposite sense to the mean interstellar field in this direction.

The short timescale of the changes suggests that the region concerned must be relatively small. One possibility is that the changes occurred within the pulsar magnetosphere itself. There is, however, good evidence that little or no Faraday rotation occurs within this region[6]. We therefore assume that the changes are taking place within the interstellar medium. The scale of the region may be estimated from the relative velocity of the pulsar and the medium. It is likely that the highest relevant velocity is that of the pulsar; observations of pulsar proper motions[7,8] show that pulsars typically have velocities in excess of 100 km s^{-1}. The offset between the position of PSR 0833−45 and the centre of symmetry of the Vela supernova remnant together with the pulsar characteristic

Table 1 Rotation measure, dispersion measure and intrinsic position angle for PSR 0833−45

Date	Observing frequencies (MHz)	Rotation measure (rad m^{-2})	Dispersion measure (cm^{-3} pc)	Intrinsic position angle (°)	Reference
May 1970	300, 410, 467 628, 1410	33.6±0.1	69.08±0.01	63.1±1.5	2, 3
December 1971	338, 395–400	33.9±1.5	—	—	Present work
July 1975	631–649, 1612–1720	37.0±0.17	68.99±0.13	63.5±5.7	Present work
June 1976	268–278, 631–648	38.17±0.06	68.53±0.3	65.2±1.5	Present work

age suggests that the Vela pulsar may have a transverse velocity as large as 500 km s^{-1}. If we adopt this figure as a reasonable upper limit to the velocity, an interval of 6 yr corresponds to a transverse scale length of 3×10^{-3} pc or 600 AU for the electron density and field fluctuation. If we further assume that the line-of-sight dimension of the region is comparable, then the excess electron density corresponding to a dispersion measure change of ~ 0.20 cm^{-3} pc is about 65 cm^{-3}. Of course, if the region has smaller dimensions the electron density would be greater.

Since this electron density is high relative to normal interstellar values we can approximate the weighted mean line-of-sight component of the magnetic field in the time-varying region by

$$\langle B_1 \rangle = 1.23\, \Delta(\mathrm{RM})/\Delta(\mathrm{DM})$$

where Δ(RM) and Δ(DM) are in their conventional units and $\langle B_1 \rangle$ is in microgauss. For $\Delta(\mathrm{RM}) \sim 4.5$ rad m^{-2} and $\Delta(\mathrm{DM}) \sim 0.2$ cm^{-3} pc we have $\langle B_1 \rangle \sim 30$ μG. This estimate will be too high if the electron density in the region concerned is larger than that assumed, either because of an underestimate of the dispersion measure change or because of the presence of a non-varying component. The estimate will be low if the dispersion measure change is less than that assumed.

It is well known that supernova remnants contain filaments of enhanced optical and non-thermal radio emission. The small indicated size of the time-varying region, together with the relatively strong magnetic field and high electron density values derived above, suggests that the observed changes result from the motion of a filament in front of the pulsar. The derived parameters are well within the range of those found for filaments in supernova remnants. For example, Duin and van der Laan[9] deduce that filaments in IC 443 have typical dimensions of 10^{-3} to 10^{-2} pc, magnetic field strengths of 150 μG and electron densities of 250 cm^{-3}. Bell *et al.*[10] deduce even larger field strengths for regions of intense radio emission in Cassiopeia A, about 3,000 μG. An Hα survey of the Vela supernova remnant by Elliott *et al.*[11] showed no evidence for an intense filament in the vicinity of the pulsar. However, diffuse nebulosity was detected in this region. The present results suggest that this nebulosity contains small-scale structure and regions of enhanced magnetic field.

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Observational consequences of synchrotron self-Compton models of compact extragalactic X-ray sources

RECENT measurements[1-6] have confirmed the existence of a class of extragalactic X-ray sources quite different from those associated with clusters of galaxies. These sources (Cen A, NGC4151, NGC1275, 3C390.3 and 3C273) are characterised by flat X-ray spectra, low-energy X-ray absorption, strong optical emission-line spectra and a compact radio or millimetre component[4]. These observations give strong impetus to the construction of models[7,8] in which these phenomena are related. We report here some of the results of a synchrotron self-Compton model[9] (SSC) which we feel can account for many of these observational properties. This model includes the effects of adiabatic expansion and synchrotron losses. We concentrate here on two of the three most studied sources—NGC4151 and NGC1275—and report our results on Cen A elsewhere. We use the results of this analysis to make tentative observational predictions about the colours of the nuclei of potential X-ray sources.

In this SSC model the non-thermal radio, X-ray, and optical emission originate in an extremely small region located in the nucleus of the galaxy. The non-thermal flux causes photoionisation of the gas in this nucleus which results in the observed strong emission-line optical spectrum. In addition this gas absorbs low-energy X-rays causing a turnover in the X-ray spectrum.

We can determine several physical properties of this emitting region, assuming X-ray emission due to SSC radiation, that is, that X rays are produced by the Compton scattering of lower energy synchrotron photons off the radiating population of relativistic electrons. Two relations between observables are[7]:

$$B \approx 7.42 \times 10^{-60}\, i_{ao}^{2}\, \nu_n^{5}\, \theta_s^{4}\, F_{\nu_n}^{-2} \text{ gauss} \qquad (1)$$

and

$$F_{\nu_x} \approx F^2_{\nu_n} (\nu_x/\nu_n)^{-\alpha}\, \nu_n^{\alpha}\, \theta_s^{-2}\, B^{-(1+\alpha)} \qquad (2)$$

$$[2.75 \times 10^4\, e_{ao} (2.79 \times 10^6)^{-(1+\alpha)} \ln \Lambda]\ \text{erg cm}^{-2}\ \text{s Hz}$$

where F_{ν_x} is the X-ray flux at the frequency ν_x, θ_s is the angular size of the source (radians), B is the magnetic field in the emitting region, F_{ν_n} is the radio or millimetre flux at the synchrotron self-absorption frequency ν_n, and i_{ao}, e_{ao} and ln Λ are functions of order unity discussed by Jones *et al.*[7]. We have assumed isotropic disordered magnetic fields and no reisotropisation of pitch angles.

Assuming that the hard $E^{-0.4}$ energy X-ray spectrum of NGC 4151[1,10], which exists out to at least 100 kev, originates by the SSC process in a component for which ν_n is in the millimetre band, as is required by recent upper limits (M. Werner 1976, H. Hudson and T. Soifer 1975, personal communications), we determine that $\theta \approx 1.4 \times 10^{-5}$ arc s (~ 1.5 light d at 19 Mpc (ref. 11)), $B \approx 2$ G, and that the synchrotron lifetimes of electrons responsible for the 2–10 keV X rays is ≈ 7 d. Here we neglect any effects due to adiabatic expansion. The model predicts that a sudden injection of a large number of relativistic particles would result in an X-ray flare for which the rise and decay times would each be a few days. The time scale of flux increase is that of the filling of the emission region by relativistic particles; the time scale of decay is that of synchrotron energy losses. We expect the X-ray spectrum to steepen with both time and energy during the decay period, so that the length of an observable flare should be shorter at higher X-ray energies. The X-ray spectral index should be

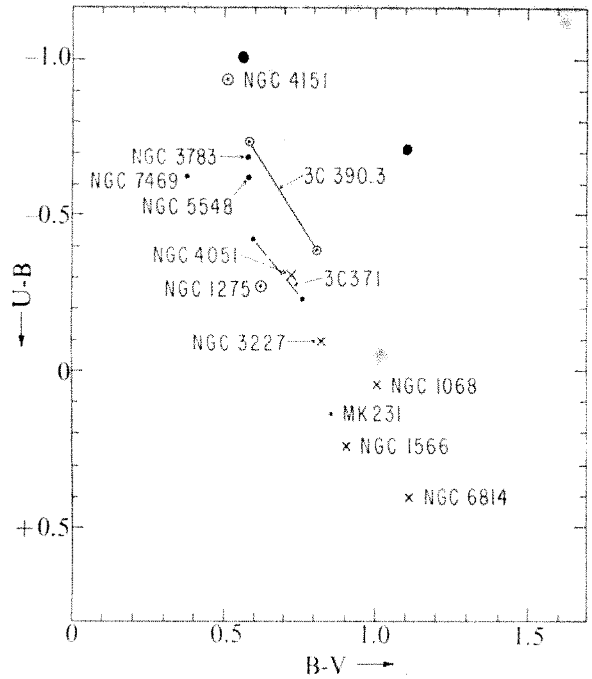

Fig. 1 The colour–colour plot for the nuclei of selected active galaxies. The lines represent the various colours seen in the nuclei of 3C390.3 and 3C371. The crosses mark those sources shown not to have detectable X-ray emission, the circles those known at this time to emit X-rays, and the dots are objects unobserved in the X-ray. The relative contribution of non-thermal radiation to the optical flux of a galactic nucleus increases from lower right to upper left in the U–B, B–V plane.

about the same as the radio (millimetre) index up to a high frequency radio break. Above this break frequency, the source should produce a power law infrared (IR) and optical flux with index $\alpha_{IR} \approx (4\alpha_{X\text{-ray}} + 3)/3$, where $\alpha_{X\text{-ray}} = \alpha_{mm}$ is the X-ray energy index. Since $\alpha_{X\text{-ray}} \approx 0.39$ (Ives *et al.*[2]) we expect $\alpha_{IR} \approx 1.52$, which is close to our $\alpha_{IR} \approx 1.46$ power law fit to the 2.2–21 μm data of Rieke and Low[12].

This model predicts a detectable flux of ~ 10 Jy at 300 μm from NGC 4151, that there should be no correlation between the MHz radio and X-ray flux, and that the X-ray absorption should be due to material in the forbidden line and broad-emission-line regions of the optical nucleus (ref. 2 and G. Shields and R.M., unpublished).

A similar model can be developed for NGC 1275 using the recent radio data of Pauliny-Toth *et al.*[13]. Here observations give $\nu_n \sim 8.5$ GHz, $F_{\nu_n} \sim 71$ Jy and $\alpha_{radio} \sim 0.45$ if we assume a single, extremely compact component. To explain the measured X-ray flux from the core of the Perseus cluster[19], presumed to be due to the nucleus of NGC 1275, we derive $\theta_s \sim 1.4 \times 10^{-3}$ s (2.1 light years at 100 Mpc), $B \sim 0.1$ gauss, synchrotron lifetimes of ≈ 10 y and $\alpha_{X\text{-ray}} \approx 0.45$. The measured VLBI[13] angular size is $\approx 1.3 \times 10^{-3}$ s and Burbridge *et al.*[14] find a typical time for variability in the compact radio component of 12 yr. We expect a non-thermal IR and optical flux with spectral index $\alpha_{IR} \sim 1.6$ which agrees well with a power law fit to the aperture-corrected data of Penston *et al.*[15], for which $\alpha \approx 1.56$. It is also expected that NGC1275 should show absorption in its X-ray spectrum and that there will be a correlation between X-ray emission and the intensity and turnover frequency of the GHz radio emission.

In this SSC model the non-thermal optical and infrared flux in the nuclei of these objects is related to the X-ray flux. Thus there should exist a correlation between the relative strength of the non-thermal component in the nucleus of a galaxy and the probability of X-ray emission from it. Sandage[16] has shown a relationship between the position of a nucleus in the U–B, B–V plane and the strength of the non-thermal component. In Fig. 1 we show the colour–colour diagram for a sample of well studied low redshift active galaxies. Following Sandage, the colours of the nuclei[16–18] of these galaxies have not been corrected for reddening. We note that all those known as of June 1976 to have X-ray emission (NGC4151, NGC1275, 3C390.3) lie in the part of the diagram corresponding to strong non-thermal components while those objects known not to emit X rays[19] (NGC1068, NGC1566, NGC3227, NGC4051 and NGC6814) lie in a region corresponding to weak non-thermal components. Based on this correlation we suggest that a search be made for X-ray emission from NGC3783, NGC5548, NGC7469 and 3C371.

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Absolute scale of thermoelectricity

I HAVE obtained the absolute thermoelectric power of lead between 10 and 350 K by direct measurement of the Thomson heat for the first time. Above 20 K the values are higher than the present scale by up to 0.30 μV K⁻¹. Acceptance of the new values would require the recalculation of the thermopowers of almost all metals, since lead was almost certainly the reference material for the thermocouple measurements which were used to determine them. The size of the correction makes it particularly significant for the theoretical interpretation of the thermopowers of many metals and alloys.

The presently accepted scale of the thermopower of lead is that of Christian, Jan, Pearson and Templeton[1]. They calculated the thermopower S from the voltage V of a lead against superconductor thermocouple and from the Thomson heat μ of lead of Borelius and coworkers (ref. 2 and many earlier papers), as

$$S(T) = \int_0^{T_1} \frac{d^2V}{dT^2} dT + \int_{T_1}^{T} \frac{\mu}{T} dT \quad (1)$$

where T_1 was about 17 K. In fact the Borelius values for μ were obtained indirectly from measurements of the Thomson heat of an alloy and the thermopower of lead relative to the same alloy. The Borelius measurements were made more than 40 years ago and have never been tested below 100 K by comparison with direct measurements of the Thomson heat.

In the present work two identical lead wires were placed in a small temperature gradient ΔT and a direct current i was passed through them (going up the gradient in one wire, down it in the other). The change in temperature δT at the midpoint of one wire on reversal of the current direction was measured by means of a differential thermocouple. (By symmetry, δT is half the change of the temperature of the midpoint of one wire with respect to the temperature of the midpoint of the other wire.) From the general solution given by Nettleton[3] for the temperature distribution in the wire, it can be shown that the Thomson heat can be calculated from

$$\mu = 4kA\,\delta T/iL\,\Delta T \qquad (2)$$

where k is the thermal conductivity; A, the cross-sectional area; and L, the length of each wire. A full description of the experiment will be published later.

The present values of μ/T agree within 5% with the superconducting experiment[1] from 10 to 17 K. The values are several times larger than the earlier values from 20 to 40 K, reconverge at 80 K, and stay within 10% of the earlier values to 290 K.

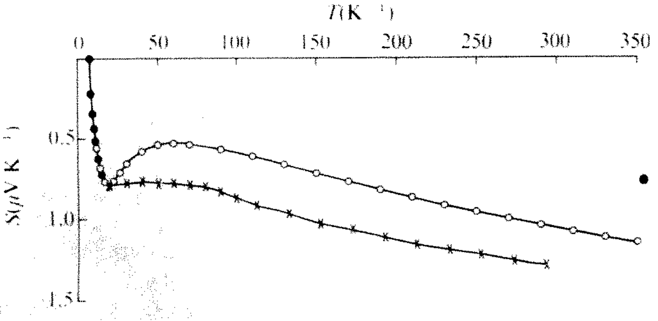

Fig. 1 Absolute thermopower of lead. , Present result; ●, Christian *et al.*[1] thermocouple experiment; , Christian *et al.*[1] compilation of Borelius[2] measurements.

The small region of mismatch of μ/T has a large effect on the thermopower scale. In Fig. 1 the present results have been tied to the old scale at 17 K. The agreement in the overlapping region from 10 to 17 K is within 0.02 μVK⁻¹. Above 17 K the two scales diverge rapidly to be 0.24 μVK⁻¹ apart at 50 K. This difference is approximately maintained at higher temperatures. The new curve is much smoother than the old, and it resembles the thermopower curves of other pure metals, having a well defined phonon drag peak. The thermopower of lead and the difference ΔS between present work and that of Christian *et al.*[1] are given in Table 1. Linear interpolation into ΔS can be used to transform thermopowers on the old scale to the new scale within 0.01 μV K⁻¹.

The measurements on lead were confirmed by the following experiments. The thermopower of a silver–1.2% gold alloy was calculated from 15 to 290 K from measurements on a lead-alloy thermocouple using the new curve for lead. The Thomson heat of the alloy was also measured directly from 15 to 290 K and its thermopower calculated by integrating equation (1) upwards from the thermocouple result at 17 K. The thermopowers agreed within 0.02 μV K⁻¹ over the whole range (see Fig. 2). This test is particularly sensitive to the effect of systematic errors in the Thomson heat measurements because, at most temperatures, the Thomson heat of the alloy is of opposite sign to that of the lead. Any significant heat loss from the sample (the most probable source of error) will cause the magnitude of the observed μ to be too small in each case. The errors are therefore additive. Only if both samples lose no heat will the thermopowers calculated from the Thomson heat results agree with those observed in the thermocouple experiment. These experiments confirm that the new scale is correct within 0.02 μV K⁻¹.

Table 1 Preliminary values of the thermopower of lead and correction to scale of Christian *et al*[1].

T (K)	S (μV K⁻¹)	ΔS (μV K⁻¹)
17	−0.78	0.00
20	−0.78	0.00
25	−0.72	0.06
30	−0.66	0.12
40	−0.58	0.19
50	−0.54	0.24
60	−0.53	0.25
80	−0.54	0.25
100	−0.58	0.28
150	−0.71	0.30
200	−0.83	0.29
250	−0.95	0.26
300	−1.05	—
350	−1.14	—

$\Delta S = S_{Pb}^{Roberts} - S_{Pb}^{Christian}$. A more closely spaced table will be published later.

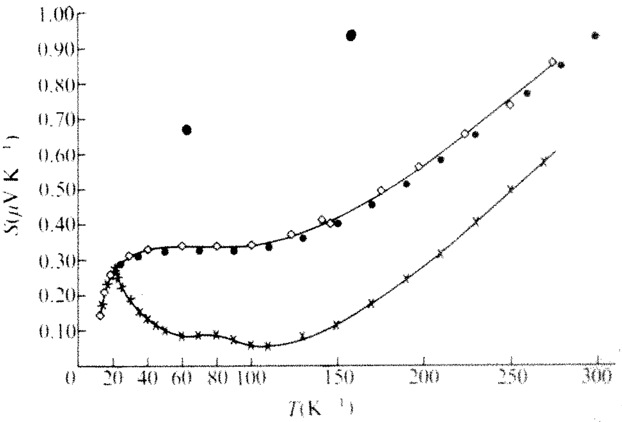

Fig. 2 Absolute thermopower of silver–1.2% gold alloy. ●, From direct measurement of Thomson heat; ◇, thermocouple experiment: Roberts scale for S_{Pb}; ×, thermocouple experiment: Christian *et al.* for S_{Pb}.

Figure 2 also serves as an example of how correcting thermopowers by means of Table 1 can be expected to produce drastic changes in the thermopowers of some alloys. On the Christian *et al.* scale the phonon drag peak for the silver–gold alloy is very narrow. On the new scale it appears very broad indeed, possibly decaying as T^{-1} to room temperature.

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Fission/α analysis of the U, Th families

A MODIFICATION is presented here of the technique of particle track analysis (PTA) by means of which it is possible to measure in particular geologic materials not only uranium but also thorium and/or radioactive disequilibria in the U and Th families.

PTA based on slow-neutron-induced fission is by now a standard method for U analysis (ref. 1, and references therein), but the technique has not been so successful for Th. Methods have been proposed based on the fission of Th by various energetic particles[2–5], but few actual analytic studies have been carried out on geologic materials, probably because of the difficulties associated with providing fluxes of such energetic particles which are both sufficiently large and known with sufficient accuracy.

The method proposed here is based both on the slow-neutron fission of U and on the natural α-decay of U, Th, and their radioactive daughters. Cellulose nitrate (CLN) records α-tracks, as other plastics such as Lexan record fission tracks[6]. If a flat surface of a U, Th-bearing material is placed against a sheet of

Table 1 Th analyses of known geological materials: *U* determined by fission track analysis. *Th* according to equation (1)

Sample	*U* (p.p.m.)	R_u	$R_{obs.}$	R_{th}	*Th* (p.p.m.)	*Th* (other work)
1. Spergen (limestone)	1.1	0.00249	0.00253	4×10^{-5}	0.06 ± 0.06	0.1 ± 0.1†
2. NBS-611* (Glass)	462	0.174	0.346	0.172	410 ± 50	455 ± 3.6‡
3. NBS-613* (Glass)	37.5	0.014	0.026	0.012	29 ± 7	37.55 ± .09‡
4. Tektite 1	2.1 ± 0.6**	0.00479	0.0134	0.00861	12.1 ± 2.2	13.0 ± 2.3**
Tektite 2 (Indochinites)	2.1 ± 0.6**	0.00479	0.0131	0.00831	11.7 ± 2.2	13.0 ± 2.3**
5. Enkaf 1 (Mn-rich deposit)	4.1	0.0102	0.0106	0.0004	0.5 ± 0.5	0.68 ± .01††

*Corrected for disequilibrium due to isotopic fractionation and $T < T'$
† H. Rydell, personal communication, 1972 (α-spectrometry)
‡ Ref. 7
** Ref. 10 (Indochinite mean)
†† Ref. 11

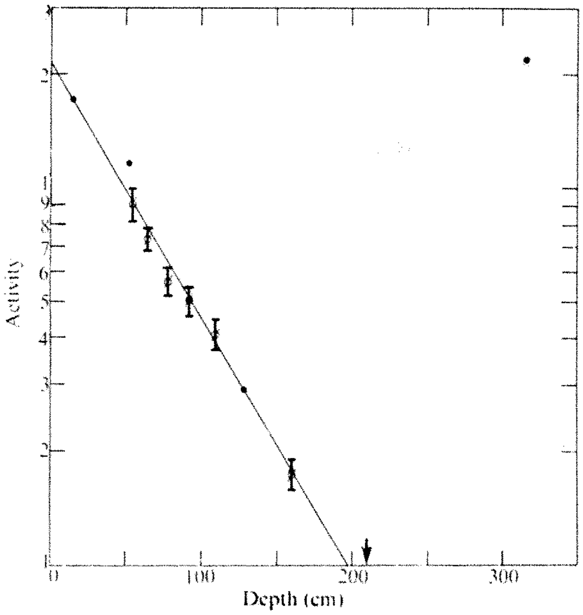
CLN, naturally-occurring α-particles from the U and Th families will impinge on the CLN and leave detectable tracks. The total α-activity of the sample is thus measured. The sample (a disk of pressed powder) is then placed in contact with a sheet of Lexan and irradiated by slow neutrons, for normal fission track analysis of the U alone.

The density of α-particle tracks in the CLN is given by $\rho = Rt$, where R is the rate at which α-tracks are produced per minute per cm² and t is the time the sample is in contact with the CLN. R is given by

$$R = W^{1/2}(k_u U + k_{th} Th) \qquad (1)$$

with W the atomic weight calculated on the atomic fraction basis; k_u and k_{th} constants depending on the decay constants, α-ranges, and counting criteria; and U and Th indicating concentrations in p.p.m. This expression based on the empirical Bragg–Kleeman relation, was first published by Cherry[7], who used it to describe the total α-particle activity measured by thick-sample scintillation counting techniques. I have determined the k-values for the present work by measuring the density of tracks produced in CLN exposed to materials of widely-differing W: U metal foil, ThO$_2$ powder, and artificial glasses with known contents of U and Th[5,8]. These values will vary depending on the individual investigator's criteria for identifying tracks in the CLN; my values are $k_u = 5.15 \times 10^{-4}$, $k_{th} = 1.6 \times 10^{-4}$.

Fig. 1 α-Activity in core V19-61. ●, Excess ^{230}Th activity in d.p.m. g^{-1} (ref. 9). ○, R values, normalised at 93 cm depth.



With the U content of the sample given by the fission track analysis, the Th content can be calculated from equation (1). Table 1 shows the results of some determinations on a variety of geological materials of known U and Th contents.

The time t necessary for the accumulation of α-tracks is a function of the U, Th contents and the accuracy desired. Densities of 100 tracks cm^{-2} are easily measured, but one will normally desire at least several hundred tracks for statistical considerations. Samples with $U = 1$ p.p.m. and $Th/U = 4$ will generate densities of 500 tracks cm^{-2} in about two months. The uncertainty in the determination of Th in these conditions is about 8%.

This method of Th analysis is valid only for those samples in which the U and Th families are in radioactive equilibrium, since it is the total α-activity of the sample that is measured. This is an important but not too severe a geologic restriction. Perhaps even more important are certain types of sample which do not meet the conditions of radioactive equilibrium. Those samples which have lost or gained any of the radioactive daughters gradually or non-quantitatively, by such processes as weathering or migration, must simply be discarded, but there are certain geological processes which establish radioactive disequilibria in precise conditions, and these may be investigated by this technique.

One particular example is the deposition of deep-sea sediments, in which an excess of ^{230}Th over that established by radioactive decay from ^{238}U is deposited, owing to the much lesser solubility of Th than U in water. As the sediment is buried by succeeding debris, this ^{230}Th (being unsupported by any longer-lived parent) decays away with its characteristic half life of 7.5×10^4 yr until equilibrium is established at a lower activity level by the U present. The decay of excess ^{230}Th is one means of establishing deep-sea sedimentation rates[9]. Other nuclides in the U and Th families may also be deposited out of equilibrium, but previous studies have shown that ^{230}Th is much the dominant species.

Measurements of the total α-activity in such sediments, corrected for α-activity in equilibrium with the U present, should show this characteristic decline with depth unless significant postdepositional mobility of members of the U, Th families should separate the various components of the total α-activity and thus disturb the correlation. Figure 1 shows the results of such an investigation into core V19-61 collected by the Lamont Observatory from near the East Pacific Rise, for which an excess ^{230}Th chronology has been established[9]. The straight line is the best fit through the ^{230}Th points: clearly the total α-activity data show the same slope, and therefore the same sedimentation rate as that obtained by direct measurement of the ^{230}Th.

The advantage of the fission/α-particle (f/α) technique is its simplicity and rapidity. Although experimental data on deep-sea sedimentation rates are of extreme value, there are very few such data in the literature because of the laborious procedures involved in the wet chemistry and α-spectrometry necessary for ^{230}Th measurements. On the other hand, all the f/α data of Fig. 1 represent one day's work. General applicability of the f/α method to this problem will depend on the question of postdepositional mobility of the U. Th families in deep-sea sediments; the lack of such mobility observed in core V19-61 may not be a common feature of ocean basins. The ease of the method will greatly facilitate the necessary surveying studies.

Many similar geological situations exist, in which the f/α method may be useful. Sediments with very high rates of sedimentation may be analysed by measuring the growth of the ^{226}Ra daughter of the excess ^{230}Th (half life 1622 yr) for the first few thousand years and then the decay of the ^{230}Th and its daughters after the Th–Ra equilibrium has been established. Corals incorporate U preferentially to Th, and so the growth towards equilibrium of ^{230}Th rather than its unsupported decay may be used as a chronometer in this case. In general, the method should be applicable to any geological situation in which a precise disequilibrium is established at a particular time, and in which the total α-decay is dominated, aside from U, by one nuclide and its daughters.

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Light-driven, carrier-mediated electron transfer across artificial membranes

WE report that light-induced electron transport across artificial membranes mediated by electron carriers has been realised by photogeneration of the reducing species.

Quinone-type carriers achieve redox processes across membranes[2–4] and are involved in mitochondrial and photosynthetic electron and proton transport (ubiquinones, plastoquinones, vitamin K)[4–6]. They perform cotransport (symport) of two electrons and two protons through their reduced hydroquinone form. Ferrocene type carriers[4] operate by way of oxidation to the ferricinium cation.

Electron transfer experiments have been conducted in a cell in which an aqueous solution of a reducing agent (RED: sodium dithionite or ascorbic acid, reduction potentials $E_0 = -1.13$ and $+39$ V respectively)[7] is separated from an oxidising aqueous solution (OX: potassium ferricyanide, $E_0 = +0.36$ V)[7] by a diphenyl ether membrane supported on a Millipore filter and containing a suitable carrier molecule. The carriers used were either menadione (vitamin K$_3$; 2-methyl-1,4-naphthoquinone, NQ, $E_0 = +0.44$ V)[7] or dibutyl ferrocene (DBF, $E_0 = +0.23$ V)[8]. The electron transfer process is followed by monitoring the reduction of ferricyanide to ferrocyanide at 420 nm, where only the former absorbs. In the absence of carrier in the membrane no reduction is observed. When either NQ or DBF are dissolved in the membrane, ferrocyanide is formed in the OX phase showing that electron transfer occurs through the NQ/NQH$_2$ and DBF$^+$/DBF redox carrier systems (Table 1). The transport rate increases with increasing concentrations of NQ in the membrane although not linearly (Table 1, runs 3–6). The absence of leakage through the membrane was checked at the end of the experiments by ensuring that no iron hexacyanide had passed into the RED compartment.

Coupling electron transport to light irradiation should be possible in a system where irradiation would generate a reducing species which would then be able to transfer an electron to a carrier molecule through the interphase, but which would not itself cross the membrane. Methyl viologen (MV^{2+}, 1,1′-dimethyl-4,4′-bipyridinium chloride) seemed to be a suitable substance; it has been used in many physicochemical and biological (for example, in photosynthesis) redox studies. Reduction of the colourless oxidising form (MV^{2+}) gives a blue cation (MV$^{\cdot+}$) ($E_0 = -0.44$ V)[7], which is able to reduce quinones like vitamin K$_3$[2] and being monocharged should remain in the water phase. Furthemore, MV^{2+} may be photoreduced in aqueous solution by a photosensitised reaction involving visible light, a sensitising dye, proflavine (PF, $E_0 = -0.73$ V; $\lambda_{max} = 445$ nm), and an electron donor, ethylenediamine tetraacetic acid (EDTA)[9].

We have used this system (PF + MV^{2+} + EDTA) as the RED phase in transport experiments with menadione as the electron carrier in the membrane and ferricyanide in the OX solution, as in previous experiments. When the transport cell is kept in the dark no change in colour or in ferricyanide concentration was observed. When irradiated with visible light from a halogen lamp the RED phase turns rapidly blue, showing that MV$^{\cdot+}$ is produced, and the ferricyanide concentration decreases. If the light is turned off, the ferricyanide concentration levels off; on reillumination it continues to drop. No change in ferricyanide concentration is observed in the absence of menadione in the membrane. These results show that light-coupled electron transport has indeed been achieved by the photoactivated process shown in Fig. 1*a*. Table 1 (runs 10–13) and Fig. 1*b* show some typical experiments.

Table 1 Electron transport by carrier molecules across an artificial membrane in chemical (runs 1–9) and light-coupled (runs 10–15) redox systems

Run	RED (mM)			Carrier in membrane (mg)	Transport rate (μM × h^{-1})
1	Asc. 5			NQ(6.8)	1.3
2	Asc. 5			—	0
3	Asc. 50			NQ(1.5)	0.4
4	Asc. 50			NQ(3.2)	1.8
5	Asc. 50			NQ(5.8)	3.1
6	Asc. 50			NQ(10.9)	5.0
7	Dit. 10			NQ(7.2)	24.7
8	Dit. 10			—	0
9	Asc. 10			DBF(7.2)	3.0
	PF (μM)	MV^{2+} (mM)	EDTA (mM)		
10*	40	1.0	20.5	NQ(6.9)	9.2
11*	0	1.0	20.7	NQ(7.2)	5.0
12*	0	0	20.0	NQ(7.3)	5.0
13*	0	0	0	NQ(7.3)	0
14*	54	1.0	20.5	NQ(7.5)	7.9
15*	0	0	20.5	NQ(7.2)	0

All experiments were performed at 25 C under argon in a cell consisting of three phases: RED, reducing aqueous solution (40 ml) membrane OX, oxidising solution (40 ml); the two aqueous solutions are stirred continuously. Membrane: 75 ± 3 mg carrier solution in diphenylether on a Millipore filter (cellulose nitrate 4 cm diameter, 500 Å holes). Carriers: NQ, 2-methyl-1,4-naphthoquinone menadione; DBF, dibutylferrocene. Runs 1, 2: OX, 5 mM potassium ferricyanide, 0.2 M potassium phosphate buffer at *p*H 7.05 in both RED and OX. Runs 3–8 and 10–15 OX, 10 mM potassium ferricyanide, 0.1 M potassium phosphate buffer at *p*H 7.0 in OX and at *p*H 6.2 (runs 3-6) or *p*H 7.0 in RED. Run 9: OX, 20 mM potassium ferricyanide, 0.1 M sodium citrate buffer at *p*H 6.37 and 1.2 mM sodium bromide in both RED and OX. Asc., ascorbic acid; Dit, sodium dithionite; PF, proflavine; MV^{2+}, methyl viologen dichloride; EDTA, ethylenediamine tetraacetic acid. In the photoreduction experiments (runs 10–15) the concentration of each component of the RED phase is indicated.

*Light source: runs 10–13: 150 W halogen lamp; runs 14 and 15: 200 mW argon laser at 476.5 nm.

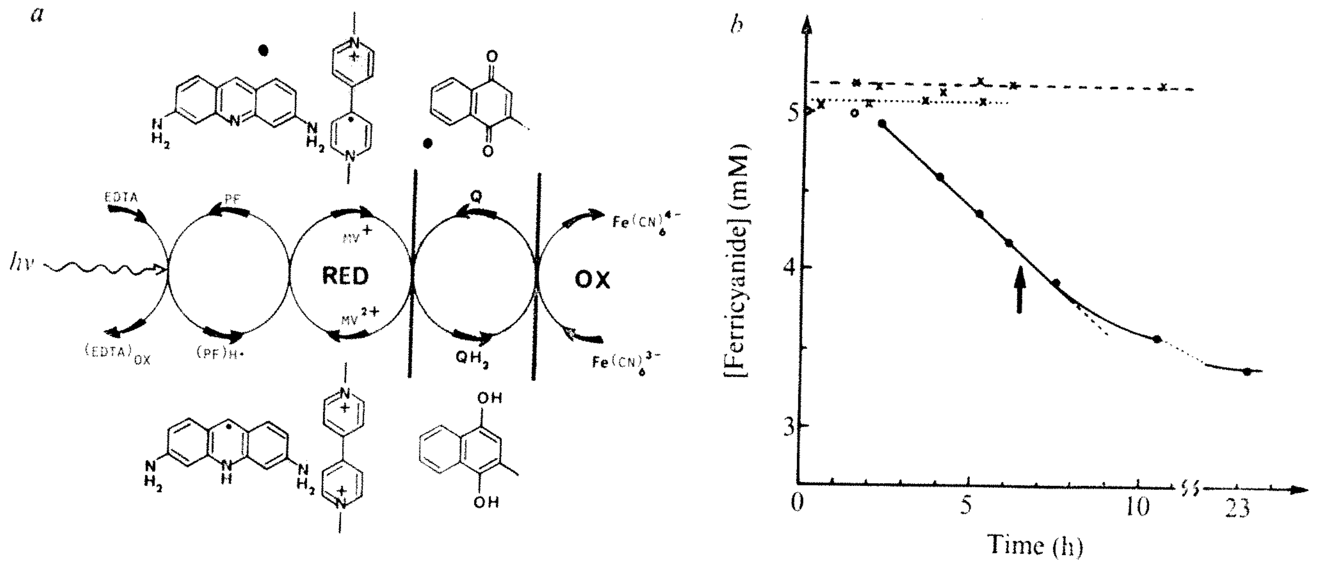

Fig. 1 *a*, Diagrammatic representation of the light coupled transport of electrons across a membrane. The reducing species in the RED aqueous phase at left is the radical cation $MV^{\cdot +}$ produced via proflavine (PF) sensitised photoreduction of methyl viologen MV^{2+} by the electron donor EDTA. The membrane contains vitamin K_3 as carrier molecule and the oxidising agent in OX is ferricyanide. *b*, Typical experiments showing the variation in ferricyanide concentration in the OX phase in three conditions: in the dark (– –); under irradiation but with no menadione in the membrane (. . .); with the complete system shown in Fig. 1*a* (—). In the latter experiment the conditions were: RED: 50 μM PF + 5 mM MV^{2+} + 10 mM EDTA in 0.1 M phosphate buffer at *p*H 6.5; membrane: 6.9 mg menadione in 72 mg diphenylether on a Millipore filter; OX: 5 mM potassium ferricyanide in 0.1 M phosphate buffer at *p*H 7.0. From the linear section of the curve the transport rate is 7.1 μM h^{-1}; the light was switched off at the point indicated by the arrow; the rest of the curve corresponds to the ferricyanide reduced by the $MV^{\cdot +}$ and the hydroquinone present at the time where the light was switched off.

The efficiency of the photocoupled electron transport (run 10) is comparable with that of the previous chemical redox systems. A problem with this system is, however, that the RED phase slowly bleaches in the course of the experiment but addition of fresh PF restores the original colour. Because bleached solutions continued to effect electron transport, control experiments were performed. The transport rate is the same in the absence of either PF alone (run 11) or of both PF and MV^{2+} (run 12), being half the rate of run 10. Thus the complete system is necessary for highest efficiency and MV^{2+} has no effect in the absence of PF. With EDTA alone, photoactivated electron transport still occurs (run 12), so experiments were conducted under irradiation with an argon laser at 476.5 nm where menadione does not absorb. The transport rate, although similar to that obtained with white light when the whole RED system is present (run 14), now becomes zero with only EDTA in the RED phase (run 15). This confirms that the naphthoquinone carrier may indeed be photoreduced by EDTA under visible light irradiation with the halogen lamp.

We have demonstrated that electron transport processes across artificial membranes using suitable carrier molecules can be coupled to light irradiation. The search for a more stable photosystem (for example metal cation complexes) may lead to applications in energy storage. On the other hand, coupling of either chemically-induced or photoactivated electron transport to cation (other than proton) symport and anion antiport may be envisaged, providing models for biological systems[5] as well as new chemical processes and potentially useful transport cells.

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Yes, Kakangari is a unique chondrite

The Kakangari chondrite seems to be the only representative so far recognised of a new class of chondrites. Mason and Wiik[1] noted a similarity between Kakangari and Mokoia (C3(V)) in bulk chemical composition when expressed on a volatile-free basis, but pointed out that the higher metal content of Kakangari suggests a relationship between it and Renazzo (C2). Recognising that Renazzo contains much less troilite and much more FeO than Kakangari, however, they concluded that Kakangari was a new type of carbonaceous chondrite, more reduced than the others. Graham and Hutchison[2] emphasised the chemical similarity between Kakangari and the ordinary chondrites, apart from its higher S content, and posed the question, "is Kakangari a unique chondrite?". Using the data of Mason and Wiik[1] as well as their own electron microprobe data, they noted that Kakangari is closer in total Fe content to the L- than the H- group chondrites but that its oxidation state lies between those of the H- and E- chondrites. Clayton *et al.*[3,4] were able to distinguish Kakangari from the ordinary chondrites on the basis of oxygen isotopes. On a three-isotope diagram, Kakangari plots with the anomalous stony-iron meteorites Bencubbin and Weatherford along an extrapolation of the apparent mass fractionation line defined by the low-temperature hydrous silicate phases of three C2 chondrites. We have now analysed a 17.1-mg piece of Kakangari for 20 elements and can answer Graham and Hutchison's question in the affirmative.

We used the INAA techniques described in previous publications from this laboratory[5,6]. The standard pot, SP[7,8], was used as a standard for most elements. Their concentrations were taken from Perlman and Asaro[8]. The

Table 1 Elemental abundances in the Kakangari meteorite

Element	Concentration (p.p.m., unless otherwise indicated)
Na	6,938 ± 8
Sc	8.70 ± 0.01
Cr	3,590 ± 10
Mn	2,700 ± 40
Fe	21.1 ± 0.1 %
Co	394 ± 1
Cu	170 ± 1
Zn	171 ± 5
Ga	9.7 ± 0.6
As	1.6 ± 0.1
Sb	0.18 ± 0.02
La	0.36 ± 0.02
Ce	1.1 ± 0.1
Sm	0.198 ± 0.009
Eu	0.088 ± 0.003
Yb	0.27 ± 0.02
Hf	0.14 ± 0.03
Os	1.13 ± 0.07
Ir	0.711 ± 0.001
Au	0.206 ± 0.001

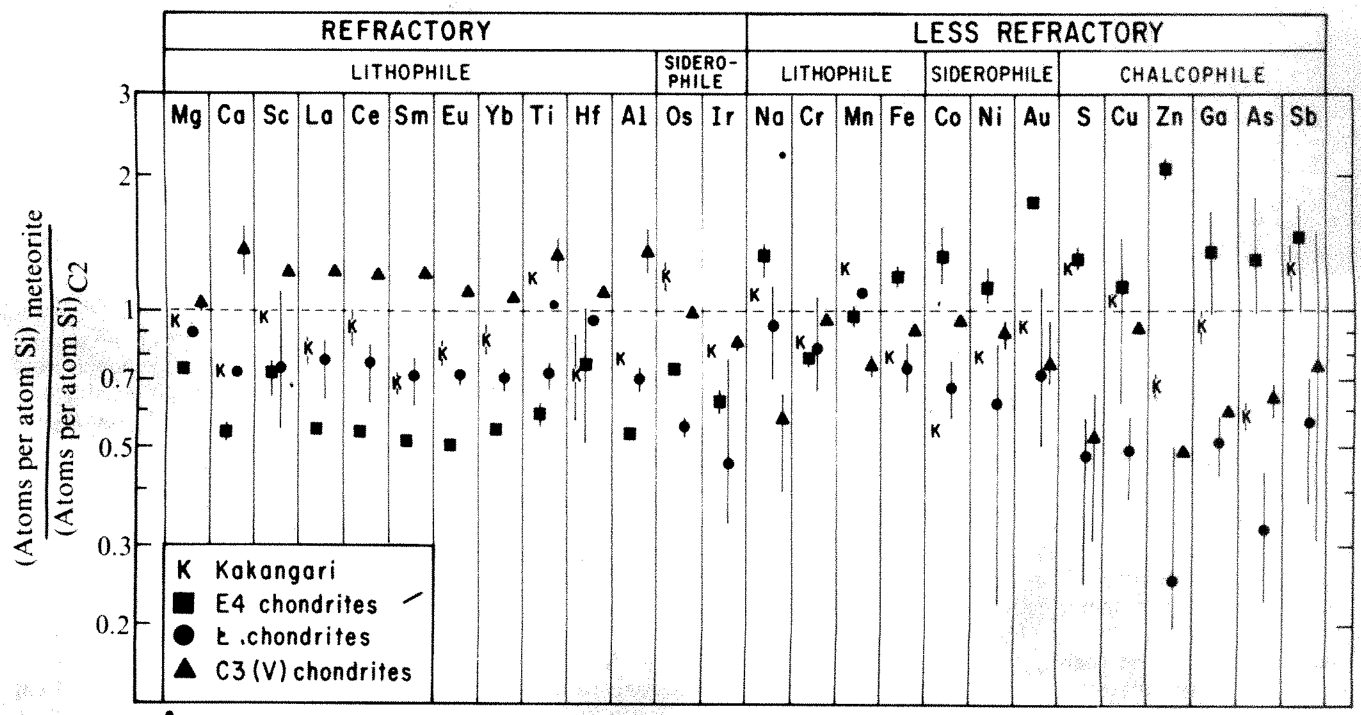
concentrations of Os, Ir and Au in Kakangari were determined from chemical standards which had been run in previous irradiations. The specific activities of ^{191}Os, ^{192}Ir and ^{198}Au at the end of the Kakangari irradiation were calculated from their values in previous irradiations by correcting for differences in neutron fluence using ^{46}Sc in SP. The results are shown in Table 1, along with errors based on 1σ counting statistics for sample and standard. Our data for Fe, Na and Mn are within 6–12% of the wet chemical data of Mason and Wiik[1]. These small differences probably reflect a small amount of sample heterogeneity. Our Cr content for Kakangari is a factor of 2.8 higher and Co a factor of 2 lower, however, than the concentrations reported by Mason and Wiik[1]. This may be due to analytical difficulties in the wet chemical determination of low concentrations of these elements. The Ti value of Mason and Wiik[1] may be similarly affected[9].

In order to compare Kakangari to other meteorite groups, all available elemental concentration data from this work and Mason and Wiik[1] were converted to silicon-normalised atomic abundances. The most similar groups, C2, C3(V), L and E4 chondrites, are represented in Fig. 1, along with Kakangari.

The less refractory lithophile and sidero phile elements are at about the same levels in Kakangari and the L-chondrites, but sulphur is enriched in Kakangari by a factor of 2.7 relative to the average L-chondrite and the chalcophile elements Cu, Zn, Ga, As and Sb are enriched by a factor of 2.1 ± 0.2 (1σ standard error). Close inspection of Fig. 1 shows also that the abundances of most refractory elements in Kakangari plot either slightly above their concentration ranges in L-chondrites or at the extreme upper limits of those ranges.

While similarities can be found occasionally in the concentration levels of some elements between Kakangari and either the E4, C2 or C3(V) chondrites, pronounced differences do exist between Kakangari and these other meteorite classes. Kakangari is higher by a factor of 1.47 ± 0.08 than E4 chondrites, lower by a factor of 0.86 ± 0.04 than C2 chondrites and lower by a factor of 0.72 ± 0.04 than C3(V) chondrites in refractory lithophile elements (all uncertainties are 1σ standard errors). Among the less refractory elements, Kakangari is higher than E4, C2 and C3(V) chondrites in Mn abundance, lower than these classes in Co content and intermediate between C2 and C3(V) chondrites in Zn concentration. Note particularly the substantial depletions of Co, Zn and As in Kakangari relative to the C2 chondrites.

Fig. 1 Si-normalised atomic abundances for Kakangari, E4, L and C3(V) chondrites, relative to C2 chondrites. Data for E4, L and C3(V) are shown with their ranges when available. The Kakangari data are shown with their 1σ error bars when the uncertainty is larger than the symbol. The data for E4, L, C2 and C3(V) chondrites are from the following sources: Na: E4, L[10], C2, C3(V)[11]; Mg, Al, Si, Ca, Ti, Mn, Fe: E4, L[12], C2[12,13], C3(V)[12,14]; S: E4, L, C2, C3(V)[15]; Sc, Cr: E4, L, C2[10], C3(V)[6]; Co: E4[16], L[17], C2[10], C3(V)[6]; Ni: E4, L, C2[18], C3(V)[19]; Cu: E4[16], L[20], C2, C3(V)[10]; Zn: E4[21], L[20], C2[22], C3(V)[23]; Ga: E4[16], L[20], C2[24], C3(V)[23]; As: E4[16], L, C2, C3(V)[17]; Sb: E4[16], L[17], C2[22], C3(V)[19]; La, Ce, Sm, Eu, Yb: E4, C2, C3(V)[25], L[26]; Hf: E4[27], L, C2[28], C3(V)[29]; Os: E4, L, C2, C3(V)[30]; Ir: E4, L[31], C2[22], C3(V)[19]; Au: E4[32], L[20], C2[22], C3(V)[19].



Our trace element data thus confirm the conclusion of Mason and Wiik[1] and answer the question posed by Graham and Hutchison[2]. Kakangari is a unique chondrite in that its bulk chemical composition does not match that of any single known class of chondrites.

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Microbiota under Antarctic ice shelves

WHETHER or not life can exist far from the open sea beneath the permanent ice shelves of Antarctica is not known, but there are three opinions. (1) A biota cannot exist at all because of the absence of surface primary and secondary productivity[1]. (2) A biota exists but with specialisations unique to life in the absence of surface productivity and in isolation under an ice shelf[1]. (3) A typical Antarctic biota exists under any Antarctic ice shelf because currents are surely present that could carry food to it[2]. Evidence supporting any of these views is unreliable. On the Ross Ice Shelf, samples taken through cracks 22 and 28 km from the open sea and in a newly calved area 8 km from the former ice front yielded rich benthic faunas but no floras[3,4]. Plant life was presumably restricted by the lack of light coming through the ice. Some of the more common species of animals at nearby McMurdo Sound were not found in the samples, indicating that there was a possibility that conditions under the ice precluded them. Such differences, however, are common in Antarctic shallow-water areas, and can be due to local substrate differences, recent grounding of ice, or sampling bias dependent on the size and penetration depth of the sampler[5]. The Ross Ice Shelf locations are so close to the open ocean that typical benthic communities might live there. At George VI Sound, marine fish whose stomachs contained several species of invertebrates were caught through a proglacial lake adjacent to the ice shelf 100 km from the nearest open water[6]. These animals were interpreted as evidence that a marine biome occurs under the entire shelf[6]. However, we describe here a microbiota from the George VI Sound occurrence that indicates that the fauna there may also be unique because it resides in a situation not typical of the entire ice shelf. Available evidence thus does not support any opinion.

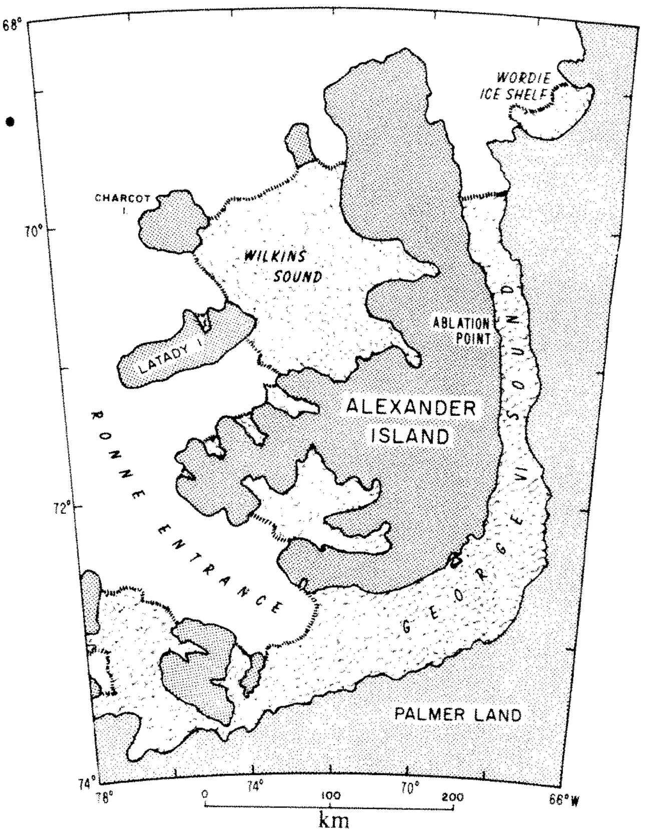


Fig. 1 George VI Sound region and Ablation Valley near the base of the Antarctic Peninsula. Ice shelves are shown in light stipple. Map from ref. 7.

George VI Sound is approximately 475 km long and 20 to 60 km wide, and is open on both ends to the Bellingshausen Sea (Fig. 1). It is covered by permanent ice varying between 100 and 500 m thick[8], but there are a few holes and cracks in it through which samples may be obtained. Mud samples collected in the proglacial lake at Ablation Valley (Fig. 1), where the fish were caught, yielded an abundant microbiota. As described by Heywood and Light[6], the lake, 5 by 4 km in size, lies adjacent to the sound and is intruded for 3.4 km

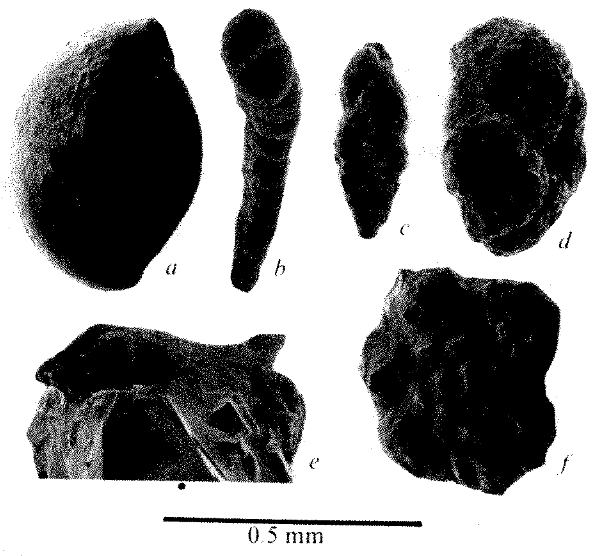
Table 1 Microbiota from two bottom samples taken in a proglacial lake at Ablation Valley, George VI Sound, Antarctica

	n	%
Diatoms		
Sample from 47-m freshwater		
Achnanthes brevipes Agardh 1824	500	100
Samples from 68.5-m seawater		
Achnanthes brevipes Agardh 1824	1	0.3
Amphora ovalis (Kützing) Kützing 1844	1	0.3
Amphora sp.	25	5.0
Diploneis spp.	5	1.0
Gyrosigma wansbeckii var. *subsalina* (H. Peragallo) Cleve 1894	1	0.3
Melosira tcherniai Manguin 1960	88	18.0
Navicula directa (Wm. Smith) Ralfs in Pritchard 1861	2	4.0
Synedra affinis Kützing 1844	42	8.0
Synedra spp.	327	65.0
Trigonium arcticum (Brightwell) Cleve 1868	5	1.0
Tropidoneis lepidoptera (Gregory) Cleve 1894	3	0.6
	500	≈ 100
Foraminifera		
Samples from 68.5-m seawater		
Conotrochammina rugosa (Parr)		
Cribrostomoides wiesneri (Parr)		
Haplophragmoides canariensis (d'Orbigny)		
Miliammina arenacea (Chapman)		
Pseudobolivina antarctica Wiesner		
Reophax sp.		
Textularia wiesneri Earland		
Tolypammina vagans (Brady)		
Gen. et sp. indet. no. 1		
Gen. et sp. indet. no. 2		

by the ice shelf. It is covered by ice, 2.5–4.5 m thick, throughout the year. Light can penetrate this thickness of ice. The lake is over 117 m deep, but only below 66.25 m is there marine water. Tidal fluctuations in the marine layer show that it is connected with the seawater of the sound[6].

The microbiota (Table 1) includes 10 species of foraminifera (Fig. 2) and at least 11 species of diatoms found in a marine sample at 68.5 m and one diatom species from a freshwater sample at 47 m. Mixing of the biotas from these water layers was not apparent in our samples. All foraminifera and diatom species are benthic. The diatoms and foraminifera are nearly perfectly preserved and quite abundant; most of the diatoms still retain some pigment. The specimens seem to have been alive or recently dead when collected. No other microorganisms possessing hard parts were found. We have found all of the species, including the fish and invertebrates, farther north in ice-free waters along the Antarctic Peninsula[5,9]. Centric and pennate diatoms have also been found in seawater present in crevasses on the Ross Ice Shelf 550 km from the open Ross Sea[10].

Fig. 2 Foraminifera from the marine layer at Ablation Valley, George VI Sound. Scanning electron micrographs. *a*, *Miliammina arenacea*. *b*, *Textularia wiesneri*. *c*, *Pseudobolivina antarctica*. *d*, *Conotrochammina rugosa*. *e*, Gen. et sp. indet. Form attached to rock fragment. *f*, *Conotrochammina rugosa*.



Our results reinforce previous conclusions[8] that an abundant and diverse Antarctic marine biota lives at Ablation Valley. Heywood and Light[6] concluded that the productivity they recorded for the freshwater layer was too low to support fish populations. The abundance of marine diatoms from lower levels, however, indicates that there is also marine primary productivity, probably sufficient to sustain higher trophic levels.

The microbiota we record from the proglacial lake can be interpreted in either of two ways: (1) it may indicate that there is indeed a diverse and rich biome under the George VI Ice Shelf as postulated[2,6], in which case it would have to be supported by a food supply carried there by currents[2] passing through the sound; or, (2) it may mean that the biota at Ablation Valley is atypical of the sound as a whole because the marine primary productivity may support a biological oasis where light penetrates through the thin ice cover of the proglacial lake. Although some diatoms perhaps could live heterotrophically under a thick ice cover, we conclude that the second possibility cannot be eliminated. Thus, in spite of a few occurrences near ice fronts and in the unique situation at Ablation Valley, there is still no unequivocal evidence of what a sub-ice shelf biome might be like.

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First Devonian platyspermic seed and its implications in gymnosperm evolution

IN the course of an investigation of fossil plants from the late Devonian rocks of Kiltorcan in southern Ireland obtained by re-excavation of the site earlier this year, we have discovered several seeds with features which contribute to the understanding of early gymnosperm evolution. The fossil flora of Kiltorcan has been known for more than a century[1], and includes the well-known and widespread late Devonian genera *Archaeopteris* and *Cyclostigma*. In addition, Johnson[2] briefly described and illustrated several oval bodies which he regarded as seeds, and to which he gave the name *Spermolithus devonicus*. His figures were at a small scale and rather uninformative, and most later workers discussing early evidence of seed plants have either been unaware of his claim, or make no reference to it. The only reference to *Spermolithus* known to us is Arnold's[3] justifiable comment that "whether it is a seed or spore case is unknown to us". In spite of the limitations of their state of preservation, we believe that the seeds we have found represent not only the earliest seed plants known from the British Isles, but also the earliest seeds with apparently platyspermic symmetry. In view of their closeness in age to the earliest generally accepted seed, *Archaeosperma*, of radiospermic character, we believe that this favours a more or less synchronous origin of radiospermic and platyspermic gymnosperms. This suggests that these two seed lineages have been separate since their inception, rather than having had a single evolutionary origin followed by rapid divergence within the Carboniferous.

Knowledge of early seed plants has grown significantly in the past decade[1-7], and has an important bearing on the interpretation of their early evolution. The most convincing record of a seed of Devonian age is that of *Archaeosperma* Pettitt and Beck[5] from the North American Famennian. This seed, although a compression, has been shown to have at least four integumentary lobes making it unmistakably radiospermic. Long[1,8,9], in his extensive researches into early Carboniferous (Tournaisian) seed plants, has found both platyspermic and radiospermic seeds with well preserved structure. He has suggested[4] that the platyspermic condition evolved from radial ovules with only two integument lobes, showing that in the early stages of platyspermy they retain the radial symmetry at the nucellar apex. This would be consistent with a single origin for seed plants in a radiospermic Devonian ancestor such as *Archaeosperma*. Other authors[10-11] acknowledge the possibility of a polyphyletic origin of gymnosperms. Our discovery supports such a hypothesis.

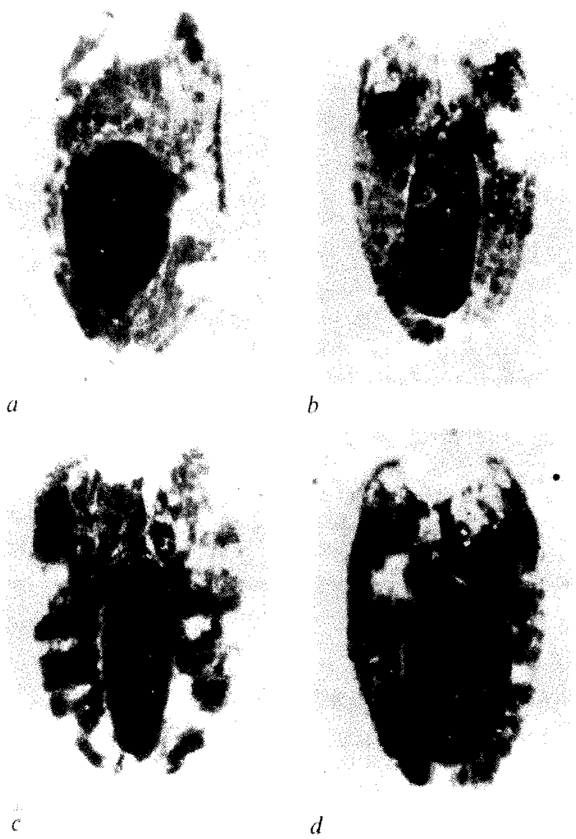

Fig. 1 Four seeds, cf. *Spermolithus devonicus* Johnson preserved as chloritised compressions from the Upper Devonian Kiltorcan Beds, Kiltorcan "Old Quarry", County Kilkenny, Eire (× 14, photographed under xylene). The assumed apical (micropylar) embayment is uppermost in each case; a basal notch is discernible, for example in (*d*). The dark central structure is interpreted as a megaspore. A possible nucellar apical expansion (or micropylar canal infill) is discernible in (*c*) and (*d*).

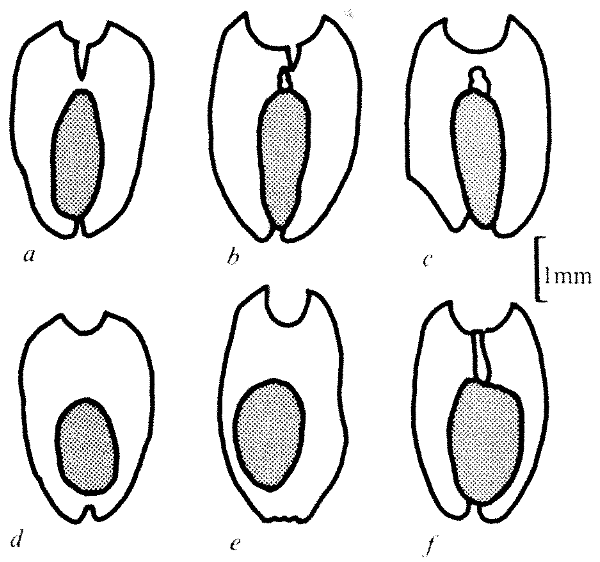

Fig. 2 Outline drawings of six specimens of seeds, cf. *Spermolithus devonicus* Johnson drawn from photographs. The scale bar is 1 mm. These show the consistent presence of the apical embayment with lateral lobes. The megaspores (stippled) show greater variation in size and shape than the enclosing structure, a characteristic feature of seed-megaspores in Palaeozoic seeds.

There are several records of seed-like bodies from the late Devonian or basal Carboniferous[12-15] of which *Condrusia minor* Stockmans[13] and *Moresnetia zalesskyi* Ananiev[16] are of particular interest. These have a distinct bicornute shape, implying platyspermy, but far too little is known about these bodies even to state definitely that they are seeds.

The discovery of the assemblage of seeds at Kiltorcan makes some contribution to this controversy of seed plant origins. The Kiltorcan fossil plant material is in an unusual, and in some ways unsatisfactory, state of preservation. The plants were evidently initially preserved as coalified compression fossils[17] in which the coaly substance was subsequently entirely replaced by a chloritoid mineral[18]. Although the resulting fossils make striking museum specimens, they are not amenable to oxidative maceration, which is the normal means of making microscopic (cuticle) preparations of fossil plant compressions. Immersion of the fossils in xylene and observation under strong incident lighting, however, reveals limited information on their internal organisation (Fig. 1).

It is critical to our case to establish that our fossils (Figs 1 and 2) are not merely seeds but that they are of platyspermic organisation. The possibility of discriminating reliably between radiospermic and platyspermic seeds, even when compressed in fossilisation, was first advanced convincingly by Arber[19]. The 30 seeds showing more or less intact outline that we recovered from Kiltorcan show a uniformity of size and shape consistent with plant propagules shed in a mature condition; in particular, they regularly show an eccentrically-placed dark oval body (stippled in Fig. 2) close to a small notch and a larger, presumably apical, embayment with two associated lobes. By analogy with better preserved seeds, we can interpret the embayment as the site of the micropyle (as in the widespread Carboniferous platyspermic seed *Samaropsis*) and the dark inner body as the megaspore (as in *Menaisperma*[20] where maceration of the inner body shows it to be a megaspore surrounded by the nucellus), and in some of our seeds there is an indication of what may be a free nucellar apex or micropylar canal (Fig. 2*b*, *c*, and *f*). This interpretation of the inner body is supported by its variation in both size and shape (Figs 1 and 2). Other workers[20,21] have demonstrated such variation in several well-preserved fossil seeds. The consistent occurrence of the apical lobes and intervening embayment rules out the possibility that we are simply seeing a random fracture plane passing through a broad micropylar opening (such as might occur in a compression state of the radiospermic *Stamnostoma*). We believe that the generally cordate form of our seeds aligns them with typical Carboniferous members of the genus *Samaropsis*, particularly *S. crassus* and *S. subacuta*[19]. On this interpretation, these Kiltorcan seeds are regarded as lying with their major transverse axis in the bedding plane as they became buried.

Provisionally, we assign our seeds to Johnson's species *Spermolithus devonicus*. His seeds, so far as can be judged from his photographs and brief description, show similar features to ours in appearing to have the oval shape, apical embayment, a central body and approximately similar size. A search for Johnson's original figured (type) material in museum and university collections in Dublin and elsewhere has been unsuccessful.

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Mouth gaping as an effective thermoregulatory device in alligators

GAPING is a characteristic activity of several species of crocodilians in which the animal lies ashore with its mouth held open for prolonged periods[1-4]. It has stirred the interest of humans for millennia: gaping is depicted in Egyptian papyri and was mentioned in the writings of Herodotus and Pliny. Today interest continues in gaping and other aspects of behaviour in these large reptiles, the last of the subclass Archosauria, the group of reptiles that included the dinosaurs[1,5]. Gaping is a complex habit that involves variable degrees of mouth closure and sometimes includes rhythmic gular movements. Behavioural observations have suggested that gaping is a thermoregulatory response[3,4] but until now physiological studies have been inconclusive. Initial reports[6] stated that gaping was effective in cooling young Nile crocodiles (*Crocodylus niloticus*) but a recent experiment on *Caiman crocodylus* indicated that this behaviour had no thermoregulatory significance[7]. Here we present evidence from controlled laboratory and field experiments that gaping is an effective mechanism for reducing head temperatures of alligators (*Alligator mississippiensis*). Gaping reduced the rate of heat gain by the heads of small and large alligators when these animals were exposed to high light radiation loads at moderate air temperatures. The magnitude of this effect increased with size. Gaping had little effect on body temperature and did not reduce the final equilibrium temperature of the head.

Head, palate and body temperatures were measured (Cu–Co thermocouples) in three alligators under controlled conditions in an environmental chamber. Alligators were anaesthetised with ketamine hydrochloride[8] and a 38-gauge thermocouple inserted in the head to a depth of 1.5 cm in the upper temporal fossa, anterior to the adductor externus profundus muscle. Palate temperature was measured with a 38-gauge thermocouple placed under the skin below the palatal fenestra and body temperature was measured with a 24-gauge thermocouple inserted through the cloaca into the colon to a depth of 10 cm. Incisions were sealed with glue (Eastman 910) and animals were allowed to recover for a period of 24 h before being tested. Each alligator was tested twice, once with its mouth closed and once with its mouth propped open at an angle of 35° to simulate the gaping posture. Alligators were placed in an environmental chamber at an air temperature in the range of 20 to 23 °C and heated by radiation and conduction until body temperature reached equilibrium. Sodium vapour lights were used to give high visible flux, and floor temperatures were controlled at an elevated level. Wind speed was 190 cm s^{-1} and radiation absorbed by the alligators was 0.76 calorie cm^{-2} min^{-1}, about that of mid-morning on a clear day.

In all three cases the heads of alligators with their mouths propped open heated at a lower rate than those of alligators with their mouths closed (Table 1). This was indicated by the determination of time constants. Time constants for the equilibrium of head temperature for the open mouth condition (τ_O) were greater than those for the mouth closed state (τ_C), ranging from 1.4 times greater for the smallest animal to 2.1 for the largest. The time constants for the rate of heating of the body (that is, cloaca) with the mouth open were similar to those with the mouth closed.

We conducted an additional experiment on a large adult (total length 2.92 m, estimated weight 100 kg) in the field. The animal was captured during October, 1975 at the Savannah River Ecology Laboratory (Aiken, South Carolina). Thermocouples were implanted as described previously and after a 24-h recovery period the alligator was placed in the sun with its mouth propped open (20° gape) at mid-morning on a clear day. The animal was not heated to its equilibrium temperature since this temperature would have been above its lethal limit. After 160 min, head temperature had risen from 23.2 to 32.7 °C and body temperature had risen from 23.4 to 26.3 °C. The next day the alligator was heated again in similar environmental conditions, but with its mouth closed. Now after 160 min head temperature went from 23.1 to 35.0 °C and body temperature rose from 23.1 to 25.2 °C. Body temperature rose 2.9 °C on the first day and 2.1 °C on the second day. This indicated that although environmental conditions seemed similar, this alligator actually had a lower net heat input to the body during the mouth closed test. Despite this fact, head temperature rose 11.9 °C with the mouth closed as compared to 9.5 °C with the mouth open. This strongly suggests that gaping was important in reducing the rate of heat gain to the head region. Thus it seems that the effect of gaping on a 100-kg alligator was similar to that on animals ranging in size from

Table 1 Temperatures and time constants for alligators heated under controlled environmental conditions with their mouths open and closed

Weight (g)	Length (cm)	Location of thermocouple	Temperatures (°C) Mouth open Initial	Mouth open Final	Mouth closed Initial	Mouth closed Final	Time constants* τ_o	τ_c	τ_o/τ_c
2,243	97.0	Head	17.4	30.2	17.4	30.4	50	35	1.4
		Body	15.9	30.7	16.8	30.6	45	40	1.1
		Palate	16.6	29.9	17.2	30.3	45	30	1.5
4,698	125.0	Head	22.4	36.2	22.2	35.9	48	29	1.7
		Body	22.2	36.4	21.8	35.3	44	41	1.1
		Palate	21.9	—	21.3	—	—	—	—
14,512	170.0	Head	23.9	32.2	23.5	32.8	145	68	2.1
		Body	18.6	31.5	19.4	31.6	144	128	1.1
		Palate	15.4	27.4	19.2	29.8	90	98	0.9

*τ_o, Time constant for mouth open; τ_c, time constant for mouth closed. A thermal time constant for a linear first-order system is the time required for the temperature of an object to travel $(1 - e^{-1})$ or 63.2% of the way to the applied temperature. The smaller the time constant the faster the temperature change. A standard heating- or cooling-curve experiment is an example of a linear first-order system. In this case body temperature changes as a function of time: $T = T_f + (T_0 - T_f)\, e^{-t/\tau}$ where T is body temperature (°C), T_0 is initial body temperature (°C), T_f is final body temperature (°C) and t is elapsed time (min). The parameter τ is the time constant. An alligator coming into equilibrium with heat sources and sinks in a complex environment can be approximated as a linear first-order system[9,10].

2.2 to 14.5 kg in weight, since rates of heat gain by the head were reduced in all cases when gaping was simulated. Gaping was an effective thermoregulatory mechanism in the environmental conditions tested in this study.

These results are expected from energy budget theory[11,12]. Exposure of the moist mucous membranes of the mouth during gaping would result in evaporative cooling. The rate of cooling would increase at low relative humidity and in the presence of a breeze. The mechanisms by which heat is transferred from the brain region to the mouth where it is dissipated during cooling are not known at present. Heat may be transferred by direct conduction through the bony skull and/or by blood circulation. Transfer of heat may also be occurring between the head and body by way of the circulatory system as has been demonstrated for *C. johnstoni*[13]. However, head–body temperature differences in reptiles can result from physical factors independent of the animal's control[14]. Therefore, definitive comments on mechanisms of heat transfer within the alligator cannot be made until further investigations of vascular patterns, conduction pathways and the mechanics of heat flow within the head have been carried out.

Though physiological studies under controlled conditions clearly indicate that gaping is a potentially useful mechanism for controlling heat gain of the head region in these animals, further studies are needed to define the importance of gaping in wild alligators. We expect that gaping would be most effective in spring, autumn and possibly winter in portions of the alligator's range in which it does not hibernate. These are times when alligators are exposed to cool water and air temperatures and should rely extensively on basking to raise their body temperature above ambient[15]. Gaping would reduce heat gain by the alligator's head and allow the animal to continue basking to allow its body temperature to continue to rise towards preferred levels. Then the alligator could retreat to the water to avoid over-heating. Gaping would be most effective in large alligators since their high heat storage capacity would require that they bask for long periods of time in order to raise their body temperature a few degrees.

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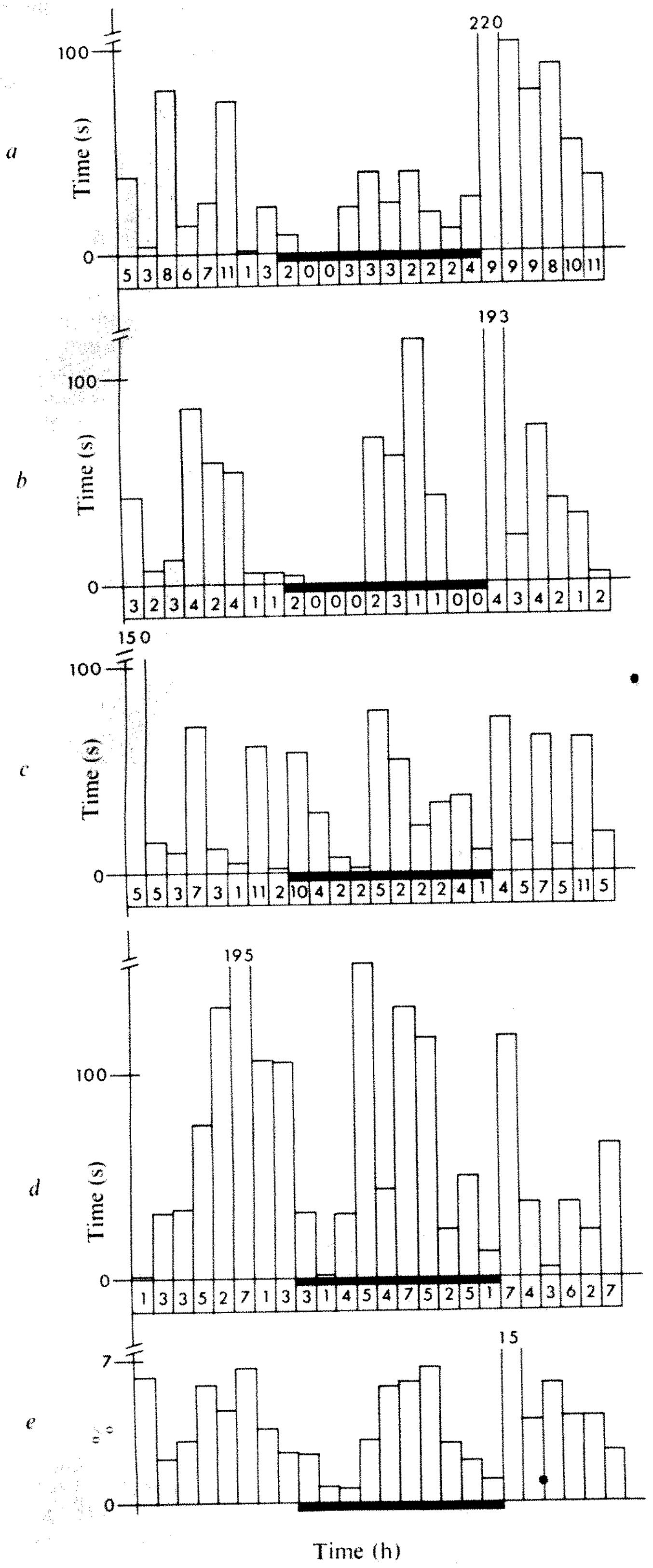
22-kHz calls by isolated rats

CALLS of about 22 kHz occur during two kinds of social interaction among rats, *Rattus norvegicus*: they are produced by submissive animals in agonistic encounters[1] and by males after ejaculation[2,3]. I have now discovered that calls of similar structure regularly occur without social stimulation and in a daily pattern controlled by the light–dark cycle. Such 'spontaneous' 22-kHz calls may help in the maintenance and development of other behaviour patterns.

The experiments reported here mainly involved round-the-clock recording of calls made by socially isolated, adult Wistar rats. Before observation each animal was entrained for at least 1 month to the light–dark schedule under which it was observed. During observation each animal was kept alone in an unused, quiet storeroom. A daily check was made on food, water and the operation of the lights, and vaginal smears were taken from the females. Calls were monitored with a bat-detector tuned to 23 kHz. This receiver produces an audible output signal in response to an ultrasonic input signal within 2–3 kHz of the frequency to which the receiver is tuned. Its output was continuously recorded on slow-moving magnetic tape which was periodically reviewed at a higher speed. Bouts of calls (groups of calls with a maximum interval between calls of 0.1 h) were replayed at the original speed, and the amount of time



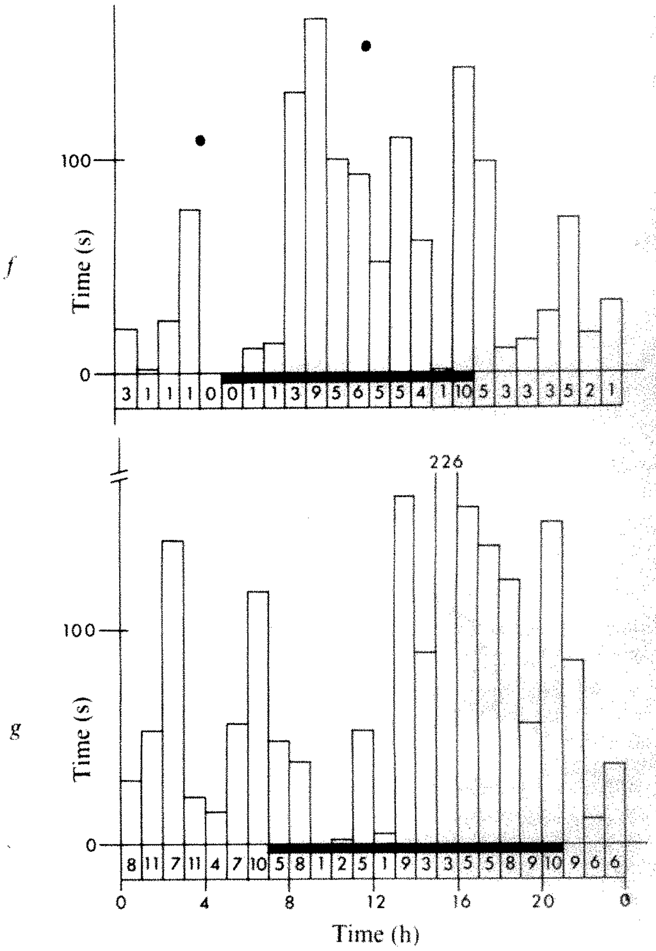


Fig. 1 Distributions of calling by six rats. Each column represents the total amount of calling(s) in that hour, summed over all days of observation. In hours in which this amount exceeded the maximum value on the ordinate, the column is topped with a number which is the amount observed in that hour. The total number of bouts in each hour and the hours of darkness are indicated below the abscissa. Hour 0 was midnight for all animals. *a*, An intact female with a 5-d oestrous cycle, observed for 20 d. *b*, An intact female with a 4-d oestrous cycle, observed for 12 d. *c*, An ovariectomised female, observed for 30 d. *d*, A male, observed for 7 d. *e*, Mean distribution of calling by animals *a* to *d* above, which were on the same 14:10 light–dark cycle; each column is the mean of the four individuals when calling for that hour is expressed as a percentage of the individual's total amount of calling. *f*, An intact female with a 4-d oestrous cycle, observed for 14 d on a 12:12 light–dark cycle. *g*, An intact female with no regular oestrous cycle, observed for 27 d on a 10:14 light–dark cycle.

spent calling was manually recorded on a cumulative timer. Sometimes the animals were also monitored with a second bat-detector tuned to 25 kHz, the output of which was recorded on a second channel of the tape recorder. This expanded the frequency range over which calls could be detected, and provided a convenient method for estimating the frequency of a call. The frequency of almost all calls was between 25 and 22 kHz. For consistency with other authors[2,3], these sounds are referred to as '22-kHz calls'.

Animals usually produced a number of calling bouts in each 24-h period. Within and between animals these bouts varied in duration (from less than a second to several minutes) and in the times when they occurred. Over a period of days, however, the distribution of time spent calling followed a pattern which was consistent for all animals regardless of their hormonal or environmental conditions (Fig. 1): the amount of calling was least in the early hours of darkness (not always the first hour), rose to a peak in the middle of the dark period, and declined towards the end of this period. When there was a 10-h dark period the decline ended abruptly in the first hour of the light period; with longer dark periods the decline ended in the last hour of darkness. There was much individual variation in calling through the rest of the light period, although there was a tendency for calling to decline gradually and then to peak again several hours before dark. Distributions *a*–*d* are highly similar ($W=0.581$, Kendall coefficient of concordance; $P<0.01$) between the onset of darkness and the peak of calling at its offset. The similarity is not as great for the entire 24-h period ($W=0.377$, $0.10>P>0.05$). The distribution of calling bouts is similar to the distribution of time spent calling (Fig. 2).

The lighting schedules of different animals were such that calling by some animals was least at hours (in real time) when calling by other animals was high. For example, in hour 18–19 (Fig. 1) calling was high for the four animals in *e*, very low for animal *f* and intermediate for animal *g*. This indicates that environmental factors other than the light–dark cycle did not influence calling.

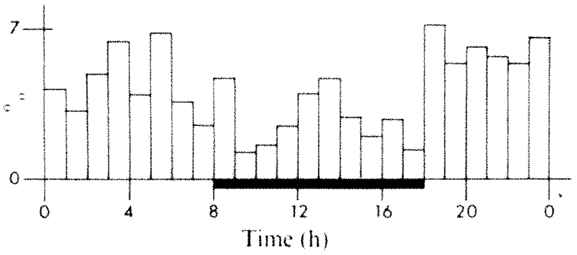


Fig. 2 Distribution of numbers of calling bouts for the four animals on the 14:10 light–dark cycle (Fig. 1, *a–d*). Each column represents the mean of the four individuals when the number of bouts in that hour is expressed as a percentage of the individual's total number of bouts.

Because stressful stimulation, such as electric shock or rough handling may also elicit calling[2], the stress of social isolation might have been responsible for the 'spontaneous' calls. But calling frequently occurs in lactating females caged with their young, in animals individually caged next to each other, and in colonies of both laboratory and wild rats (unpublished findings). During most of these latter observations there were no overt signs of agonistic or sexual behaviour, which suggests that 'spontaneous' calling is normal for grouped as well as isolated rats.

'Spontaneous' calling is evidently associated with inactivity and rest. Activity and feeding of rats is intense in the early hours of darkness, which is when calling is least. Furthermore, a number of authors report that feeding and activity then subside and later rise to a second peak a few hours before the onset of light[4–8], a common pattern among nocturnal mammals[8]. This is the obverse of the pattern in 'spontaneous' calling, the peaks of which could all be associated with times of rest when animals are preparing for, or recovering from, activity peaks during darkness. 'Spontaneous' calls may be associated with sleep. On some occasions I simultaneously observed the behaviour and calling of lactating females with their litters. Nearly always the female was apparently asleep when calling occurred. Furthermore, the EEG pattern during post-ejaculatory calling is identical to the pattern during sleep[3], and the overt behaviour of calling animals in social contexts is subdued and 'sleep-like'[1–3].

22-kHz calls in social situations follow interaction between two animals; calls by one animal seem to help pacify the other[1–3]. Since 'spontaneous' calls are not part of any such interaction, their function is not immediately apparent. Nevertheless, it is unlikely that these calls are non-functional because they are so common, because they do not occur randomly, and because they are continually produced even though they could endanger the caller in natural conditions. Many predators are probably sensitive to these calls[9], which are generally quite intense; post-ejaculatory calls can be as intense as 80 dB SPL[2]. If calls promote inactivity in conspecifics regardless of the context, their emission during inactivity could synchronise the behaviour of animals within a colony. Synchrony in the onset and ending of activity in rat colonies has often been observed, and Calhoun suggested that calls during activity may be involved. The present results suggest that calls during inactivity may be responsible. It is also interesting to speculate whether calls by lactating females play any part in forming an association in the pups between the sounds and an inactive or relaxed state.

Bell has suggested that 22-kHz calls indicate a state of decreased "arousal" and may produce a similar state in other rats[10]. The present interpretation of 'spontaneous' 22-kHz calls is consistent with both suggestions. Bell further suggests that current hypotheses about functions of rodent ultrasounds may be unnecessarily complicated because they are based on information about the structure and behavioural context of the calls. He advocates research into the physiological state of the calling animal and into the effects of calls on conspecifics. My results indicate that this approach will be necessary in future studies of 22-kHz calls in rats.

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Specific alterations in phosphorylation of cytosol proteins from differentiating neuroblastoma cells grown in culture

THERE have been a number of reports[1–10] which indicate that an experimentally induced elevation in the intracellular level of adenosine-3′,5′-monophosphate (cyclic AMP) in mouse neuroblastoma cells induces and increases the expression of many differentiated functions that are characteristic of the mature neurone. The irreversibility of cyclic AMP-induced "differentiation"[3] was attributed to the increased level of proteins which bind cyclic AMP thereby protecting the newly synthesised cyclic nucleotide from hydrolysis[11,12]. However, the mechanisms by which cyclic AMP initiates the observed morphological[1–3] and biochemical[1–10] alterations remain unclear since the activity of cyclic AMP-dependent protein kinase did not change in differentiated cells[13]. We have investigated the phosphorylation of specific proteins in control and 'differentiated' neuroblastoma cells. We report here that the cyclic AMP-dependent phosphorylation of a specific protein in the cytosol of 'differentiated' cells increased while the cyclic AMP-independent phosphorylation of another protein in this cell fraction decreased. These selective alterations may represent intermediate steps in a series of events which ultimately lead to the expression of differentiated functions.

Cells of the previously defined[3] clone NBP$_2$ were cultured and maintained as previously described[1]. Differentiation was induced by treating the cells for 3 days with 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (R020-1724)[11], a rather specific inhibitor of cyclic AMP phosphodiesterase[14,15]. Control and treated cells were homogenised in 0.25 M sucrose containing 0.05 M Tris-HCl buffer (*p*H 7.4), 3 mM $MgCl_2$ and 4 mM 2-mercaptoethanol[11,12]. The cytosol (supernatant of a 9×10^6 g min centrifugation) was diluted in homogenising buffer to contain 2 mg protein ml^{-1} (Lowry method) and stored at the temperature of dry ice until assayed. Endogenous protein phosphorylation was assayed as previously described[16]. Each of the samples studied was also tested for ^{3}H-cyclic AMP binding. Binding was found to be two-fold higher in 'differentiated' cells compared to the controls, as previously reported[11,12].

The cytosol of neuroblastoma cells revealed the presence of over 40 protein bands after the proteins of this fraction were electrophoresed in SDS gels. No differences in staining patterns were observed between samples obtained from control cells, cells that had been treated with RO20-1724, and cytosol fractions treated with the drug *in vitro* (Fig. 1). Incubation with ATP before electrophoresis also did not affect the patterns. Protein bands that incorporated ^{32}P-phosphate upon incubation were designated according to their apparent molecular weight (Fig. 1, autoradiogram). Five of these protein components (bands 55, 59, 72, 88, and 97) incorporated considerably more ^{32}P-phosphate than the other phosphorylated bands (Figs 1 and 2). Of these five major bands, the phosphorylation of one band (band 97) was not affected by exogenously added cyclic AMP. Phosphorylation of this band decreased approximately two-fold after differentiation (Table 1). The phosphorylation of the other major bands (bands 55, 59, 72 and 88) was stimulated by cyclic AMP. Addition of cyclic AMP (5 μM) stimulated phosphorylation 128–208% depending on the protein band; the highest stimulation was noted at band 59. Compared with control cells, these four major bands of the differentiated cells showed a marked increase in phosphorylation which varied from 1.2- to 2-fold. The greatest increase occurred in band 59 (Table 1). The percentage stimulation of phosphorylation caused by the addition of exogenous cyclic AMP to samples from differentiated cells was only slightly higher than in control cells. The concentration of cyclic AMP required for half-maximal stimulation (about 0.1 μM) was found to be the same in both treated and untreated cells.

In order to test whether residual drug levels in 'differentiated' cells were responsible for the changes in endogenous phosphorylative activity observed, the cytosol

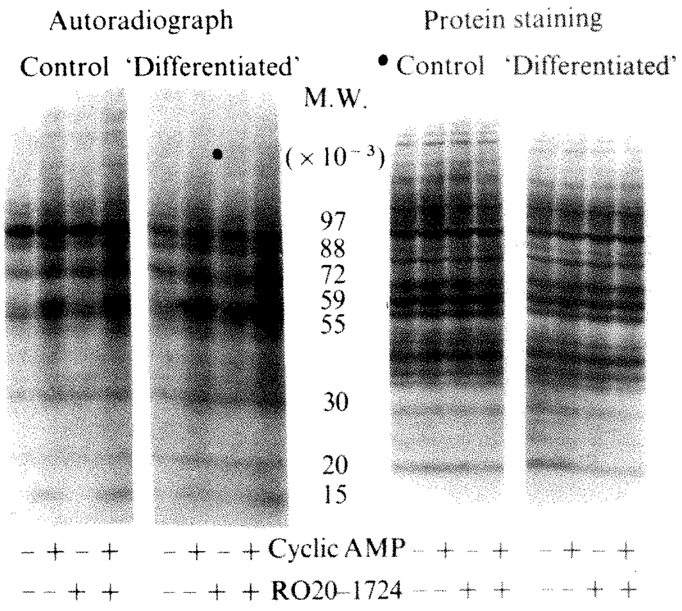

Fig. 1 Identification of endogenously phosphorylated proteins in the cytosol of control and 'differentiated' neuroblastoma cells. Standard assays of endogenous phosphorylation[20] were conducted by preincubating aliquots from the cytosol for 5 min at 30 °C. The reaction was initiated by the addition of γ-^{32}P-ATP (ICN, Irvine, California; diluted with non-radioactive Tris-ATP, Sigma, to a specific activity of 1 to 6×10^7 c.p.m. $nmol^{-1}$) with or without cyclic AMP. The reaction mixture in a final volume of 0.06 ml contained 0.66 mg protein per ml, 50 mM sodium acetate buffer (*p*H 6.5), 10 mM magnesium acetate and 3 µM ATP. After 1 min incubation at 30 °C the reaction was stopped by the addition of sodium dodecyl sulphate (SDS) solution to a final concentration of 3% SDS containing 5 mM Tris-acetate (*p*H 8.0), 1 mM EDTA, 2% 2-mercaptoethanol, 6% sucrose and 0.01% bromophenol blue which served as tracking dye. The solubilised reaction products were heated in boiling water for 2 min. Aliquots (20 µg protein) were applied to 7–14% linear gradients of polyacrylamide gels that had been polymerised in the Hoffer (California) SE-500-1.5 slab gel electrophoresis unit, and electrophoresed using SDS-discontinuous buffer system and the Ortex 4100 pulsed constant power supply. The gels were subsequently stained with Coomassie-brilliant blue-R, dried in the Hoffer SE-540 gel drier and autoradiographed by placing them in close contact with Kodak-X-Omat X-ray film. Under the electrophoretic conditions used, inorganic phosphate, ATP and lipid bound phosphate migrated more rapidly than the phosphoproteins and did not interfere with their separation. Molecular weight estimations were obtained by electrophoresis of marker proteins on the same gel slabs. The specific activity of γ-^{32}P-ATP was 3.6×10^7 c.p.m. $nmol^{-1}$ and autoradiographic exposure time was 70 h. (–) And (+) respectively refer to the addition of water or 5 µM cyclic AMP with the initiating labelled ATP. (–) and (+) also refer to the inclusion of 2 µg RO20–1724 per 60 µl (+) or solvent (–) during a 20-min preincubation period.

Table 1 Endogenous phosphorylation of five protein components in the cytosols of control and 'differentiated' neuroblastoma cells

	^{32}P-phosphate incorporated (arbitrary units)									
	Band 97		Band 88		Band 72		Band 59		Band 55	
	H_2O	Cyclic AMP	H_2O	Cyclic AMP	H_2O	Cyclic AMP	H_2O	Cyclic AMP	H_2O	Cyclic AMP
Control cells	105 ±5.2	104 ±2.4	46 ±0.5	59 ±0.7	45 ±0.5	58 ±0.9	37 ±0.7	68 ±1.0	44 ±1.9	61 ±1.8
'Differentiated' cells	60 ±2.9	63 ±2.4	58 ±4.9	69 ±1.9	63 ±2.4	77 ±1.4	66 ±3.1	137 ±2.9	61 ±3.2	83 ±3.4

R020-1724 was used to induce differentiation[11,12]. Cytosol (100,000*g* supernatant) was prepared and used in the assay of endogenous phosphorylation as described in legend to Fig. 1. Incorporation of ^{32}P-phosphate was determined by analysis of densitometric scans of the autoradiograms (Fig. 2) according to the recommendations of Ueda *et al.*[17]. The results were expressed in arbitrary density units. The results of quantitation by this method were found to be highly correlated (+0.87) with quantitation achieved by scintillation counting of corresponding gel slices[20]. Each value in this table represents the mean ± s.e.m. obtained from three separate preparations which were assayed using γ-^{32}P-ATP with a specific activity of 3.6×10^7 c.p.m. $nmol^{-1}$ in the presence or absence of 5 µM cyclic AMP. Two additional preparations were assayed with ATP of higher specific activity and gave essentially the same results. The latter were not included in the table in order to avoid undesirable normalisation of the data.

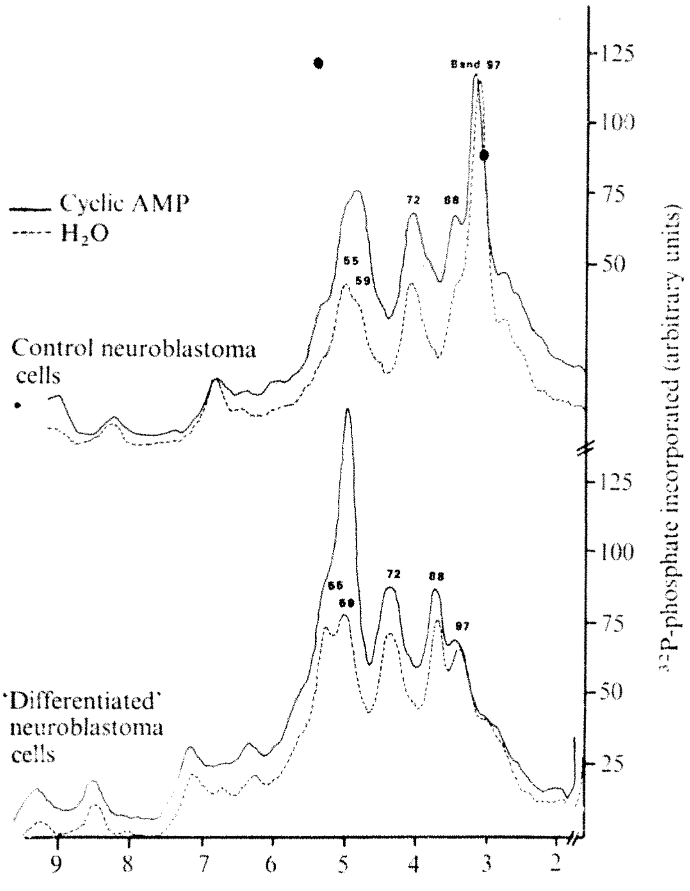

Fig. 2 Densitometric tracings of autoradiograms obtained from polyacrylamide gels after the electrophoretic separation of ^{32}P-phosphate labelled phosphoproteins of control and 'differentiated' cell cytosol. Assays of endogenous phosphorylation in the absence (— — —) or presence (———) of 5 μM cyclic AMP were carried out as described in the legend to Fig. 1. The autoradiograms were then scanned at 649 nm using a Beckman Acta CV spectrophotometer equipped with a linear transport system. Note that the pattern obtained with samples from control cells is characterised by predominance of band 97, while the "differentiated" cells are characterised by the predominance of band 59.

from control and 'differentiated' cells were preincubated for 20 min at 30 °C in the presence of RO20–1724 (2 μg per 60 μl) and the phosphorylative assay was carried out in the presence of the drug (1 μg per 60 μl). This treatment did not alter the phosphorylation patterns (Fig. 1). Thus, the *in vitro* assays of endogenous phosphorylation reflect phosphorylative modifications which had been pharmacologically induced in the intact cells.

The changes in cyclic AMP-dependent and cyclic AMP-independent phosphorylation found in the cytosol of 'differentiated' relative to control cells correlated well with the increase in binding of cyclic AMP by proteins localised in the cytosol of 'differentiated' cells. However, the question of whether the cyclic AMP-binding proteins identified in the cytosol of neuroblastoma cells[11,12] only constitute regulatory subunits of cyclic AMP-dependent protein kinases, or whether some of them can directly affect inductive processes during differentiation, remains to be answered. Preliminary observations in this laboratory have indicated that the phosphorylative alterations that occur in the cytosol were accompanied by an increase in the endogenous phosphorylation of two specific proteins in the crude nuclear fraction. Thus, the observations reported here may be relevant to mechanisms that operate in inductive processes[18].

The changes in phosphorylative activity observed in 'differentiated' cells raises several questions. Do these changes occur after the division rate has slowed down or do they act as biological signals to turn off cell division? Do the phosphorylative changes precede the induction and/or the increase in various differentiated functions[1–10] or do they follow the expression of these functions? Do these phosphorylative changes depend upon the elevation of intracellular levels of cyclic AMP or can other agents, which are known to induce some differentiation[3] without changing the cyclic AMP level, produce similar phosphorylative changes? Are phosphorylative modifications involved in the differentiation processes that occur during normal brain development? It has been suggested that cyclic AMP, in some way, increases the expression of some genes and suppresses the expression of others during differentiation of neuroblastoma cells[3,10,19]. The present findings, which demonstrate both increases and decreases in the phosphorylation of specific proteins during differentiation, lend support to these concepts. The ability of cyclic AMP to produce phosphorylative changes which persist for a long duration was previously demonstrated[20]. The present study indicates that changes of this nature may have a role in neuronal differentiation.

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Regulation of cyclic GMP in cerebellum by a striatal dopaminergic mechanism

THE cerebellum has an important function in the coordination of movement and in the regulation of posture. Neurophysiological studies have established that Purkinje cell activity is stimulated by mossy and climbing fibres, which are two distinct afferent excitatory pathways to the cerebellum[1]. Biochemically, an increase or a decrease in the activity of mossy and climbing fibres together with either an activation or inhibition of gamma aminobutyric acid (GABA) synapses of Golgi, basket and Purkinje cells results in changes of the cyclic 3′,5′-guanosine monophosphate (cyclic GMP) content of the cerebellar cortex[2–5]. Therefore, by measuring cyclic GMP in the cerebellar cortex one might infer whether drugs which influence the planning of motor activity and modify operant behaviour also change the activity of the excitatory cerebellar afferents or the function of the inhibitory intracerebellar neurones which presumably employs GABA as transmitter.

We recently reported[6] that an antagonist (haloperidol) and an agonist (apomorphine) of dopamine receptors[7,8] respectively decrease or increase the cyclic GMP content of cerebellar cortex. These changes in cyclic GMP occur without parallel modification of cerebellar cyclic 3′,5′-adenosine monophosphate (cyclic AMP) content[6]. It is currently believed that the cerebellum is devoid of dopamine receptors[9] and that the dopamine present in this structure functions as a precursor of noradrenaline[10]. Since neither cyclic GMP nor cyclic AMP content change as a result of *in vitro* application of dopamine to cerebellar slices[11] or addition of apomorphine or haloperidol to cerebellar homogenates[6], it seems unlikely that these nucleotides function in cerebellum as second messengers of dopamine receptor activation. Hence we have inferred that haloperidol and apomorphine change the cerebellar cyclic GMP content when given parenterally by interacting with dopamine receptors located in other brain areas. An area of the brain which contains a large functional pool of dopamine receptors is the basal ganglia. Changes of cerebellar cyclic GMP could then occur as a consequence of stimulation or blockade of dopamine receptors located in basal ganglia. We have obtained preliminary evidence for such a hypothesis by comparing the changes in cerebellar cyclic GMP content elicited by intrastriatal, intracerebellar and intraperitoneal injection of different neuroleptics and apomorphine.

As shown in Fig. 1, the intraperitoneal injection of haloperidol, chloropromazine and (+)-butaclamol, three drugs that presumably block dopamine receptor function[7,8], produced a dose-related decrease of the cyclic GMP content of cerebellar cortex: a 30% decrease was obtained with as little as 0.1 μmol per kg of haloperidol, 0.2 μmol of chlorpromazine and 1 μmol of (+)-butaclamol. The figure also shows that (−)-butaclamol, which fails to block dopamine receptors[8], also failed to decrease the cerebellar content of cyclic GMP. As is evident from the data shown in Table 1, haloperidol and (+)-butaclamol decreased the content of cyclic GMP in cerebellar cortex also when they were directly infused into the striatum.

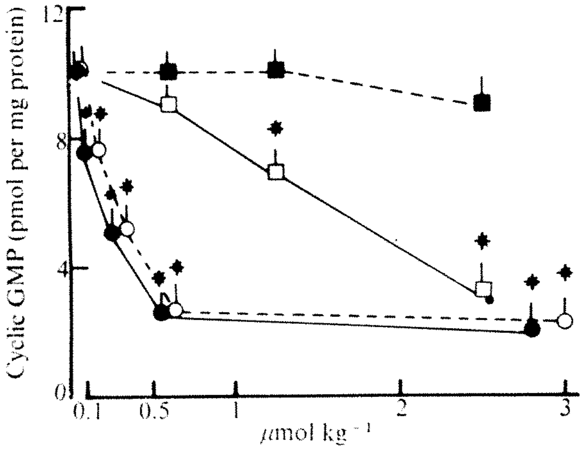

Fig. 1 Decrease in the cyclic GMP content of cerebellar cortex after various doses of haloperidol (●), chloropromazine (○), (+)-butaclamol (□) and (−)-butaclamol (■). Haloperidol, chloropromazine and butaclamol were injected i.p. 30 min before killing the rats. Male Spague–Dawley rats of 130–150 g were killed with a microwave radiation beam focused on the skull[16]. The cyclic GMP content of punches (0.8–1 mg protein) of cerebellar cortex was extracted, purified and assayed as previously reported[16]. Each point is the mean ± s.e.m. of six experiments. *$P < 0.01$ when compared with animals treated with solvent.

Table 1 Effect of intrastriatal and intracerebellar injection of haloperidol and (+) or (−)-butaclamol on cerebellar cortex cyclic GMP content

Drug	µg	Cerebellar cortex cyclic GMP (pmol per mg protein) Intrastriatal injection	Cerebellar cortex cyclic GMP (pmol per mg protein) Intracerebellar injection
Solvent		10.4 ± 4.5	9.8 ± 2.0
Haloperidol	6	4.9 ± 2.3*	10.2 ± 2.6
(+)-Butaclamol	10	5.1 ± 1.8*	10.6 ± 1.6
(−)-Butaclamol	10	9.5 ± 2.1	9.6 ± 2.4

Drugs were injected either directly in striatum (AP = 7.6; L = 2.8; DV = +1.5)[17] or in the cerebellar hemisphere (AP = 3.4; L = 3, DV = −1)[17] through chronically implanted cannulae. The intracerebral injections were made in a volume of 1 µl which was injected at a rate of 1 µl per 2 min. The animals were killed 30 min later.

*$P < 0.05$ when compared to animals treated with solvent. Each point is the mean ± s.e. of four experiments. Rats were killed 30 min after drug injection.

AP = Anterior–Posterior; L = Lateral; DV = Dorsoventral.

A 50% reduction of cerebellar cortex cyclic GMP was obtained by intrastriatal injection of 6 μg of haloperidol or 10 μg of (+)-butaclamol. In contrast, both drugs failed to change cerebellar cyclic GMP content when directly applied to the cerebellum. Moreover, the inactive isomer (−)-butaclamol did not change cerebellar cyclic GMP content after either intrastriatal or intracerebellar injection. Thus the results support the idea that the functional state of striatal dopamine receptors may be responsible for changes of cerebellar cyclic GMP.

In order to determine whether the decrease of cerebellar cyclic GMP content elicited by intraperitoneal injection of antipsychotics was mediated by a blockade of striatal dopamine receptors or by blockade of dopamine receptors located in other brain areas we studied the action of apomorphine injected intrastriatally on the cyclic GMP content of cerebellar cortex. As shown in Table 2, apomorphine (10 μg) injected in both striata increased cerebellar cyclic GMP in control animals and also completely reverted the decrease of cerebellar cyclic GMP in animals pretreated intraperitoneally with haloperidol. Hence, our results demonstrate that the content of cerebellar cyclic GMP can be influenced by activating dopamine receptors in basal ganglia.

The interaction between basal ganglia and cerebellum may occur at various levels. Striatum and cerebellum are

Table 2 Effect of an intrastriatal injection of apomorphine on cerebellar cortex cyclic GMP of rats pretreated with haloperidol

Drug	Cerebellar cortex cyclic GMP (pmol per mg protein) Solvent (1 µl)	Cerebellar cortex cyclic GMP (pmol per mg protein) Apomorphine (10 µg µl^{-1})
Solvent 5 ml kg^{-1} i.p.	9.8 ± 0.36	15.2 ± 0.21*
Haloperidol 0.14 µ mol kg^{-1} i.p.	7.5 ± 0.18*	11.1 ± 0.41†

Apomorphine was injected in both striata through chronically implanted cannulae. Rats were killed 6 and 31 min after apomorphine and haloperidol respectively. Each point is the mean ± s.e. of five experiments.

*$P < 0.001$ when compared with animals treated with solvent.

†$P < 0.02$ when compared with animals treated with 0.14 mol kg^{-1} of haloperidol.

both involved in the coordination and regulation of motor activity and operant behaviour. Both cerebellum and basal ganglia receive a substantial somatosensory input from the cortex, they filter this information and project their modulatory influence back to the cortex through a multisynaptic pathway by way of the ventrolateral talamic nuclei[12,13]. It has been proposed that cerebellum and basal ganglia act in parallel to set up patterns of thalamus cortical output which is necessary for the appropriate activation of cortico-spinal motor neurones[12]. Moreover, neurophysiological data indicate a direct link between basal ganglia and cerebellum suggesting that changes in the activity of striatal neurones can produce parallel changes in the neurones of the cerebellar cortex[14,15]. It is therefore tempting to suggest that the measurement of cerebellar cyclic GMP may be a sensitive biochemical index of the impairment of striatal function elicited by antipsychotics. These results may also be interpreted as preliminary evidence for a cerebellar role in some of the actions of neuroleptic drugs.

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Excitatory action of TRH on spinal motoneurones

The recent isolation and characterisation of a number of peptide hypothalamic-releasing (and release-inhibiting) hormones led to an unexpected bonus. Many of these peptides have been found to be widely distributed in brain in areas remote from established hypothalamic neuroendrocrine systems. For instance, two thirds of the brain thyrotropin-releasing hormone (TRH) is located outside the hypothalamus[1,2]. This finding suggests that TRH might have some neuroregulatory role in the brain besides its role as a releasing hormone. The marked behavioural effects of central origin produced by TRH support this notion[3–7]. In particular, the finding that TRH can be localised by immunohistochemical techniques to nerve fibres in a variety of brain regions[8], that it depresses neuronal activity when applied by microiontophoresis[9–11] and that it has stereoselective, high-affinity binding sites in brain[12] suggests that TRH may be a neurotransmitter. There is little evidence, however, on the transmembrane effects and mechanism of action of TRH or other peptides on central neurones.

Spinal motoneurones in the rat receive a moderate density of nerve fibres which are immunoreactive for TRH[8]. This finding provides an opportunity to analyse with intracellular recording the effects of TRH on the membrane properties of central neurones. I show here that TRH has a purely excitatory action with a potency similar to that of glutamate when applied to frog motoneurones. The action of TRH also resembles that of glutamate in that it is often accompanied by an increase in conductance of the membrane. It differs, however, from that of glutamate in that the maximum depolarisation rarely surpasses threshold suggesting that TRH provides a background facilitatory action on motoneurones. The structural requirements for this depolarising action parallel those for the release of thyrotropin[13]. Although immunohistochemical localisation studies have not been performed in frogs, particularly high concentrations of TRH are found in extrahypothalamic tissue[1].

Rana pipiens were chilled on ice and the spinal cord removed, hemisected and placed in a sucrose gap chamber. The activity of motoneurones was recorded by placing a filament of the ventral root across the sucrose gap and the potential difference across the gap was monitored with two calomel electrodes. An upward deflection indicated that the bath electrode was negative relative to the root electrode, signifying a depolarisation of the motoneurones. The cord was perfused with an oxygenated (100%) Ringer solution of the following composition: 114 mM NaCl, 2 mM $CaCl_2$, 2 mM KCl and 10 mM Tris buffer, *p*H 7.3. The temperature was maintained at 18 °C. For intracellular recording glass microelectrodes with tip resistance between 15 and 20 MΩ were selected. Motoneurones were identified by antidromic invasion and only those cells with stable membrane potentials of −65 mV or more were selected for study. The responsiveness of the motoneurone pool was continuously monitored with sucrose gap recording during the intracellular studies. Responses were recorded on a chart recorder and also photographed from the oscilloscope screen. TRH was purchased from Beckman Instruments, Peninsula Laboratories, and Calbiochem Laboratory. All samples gave similar results.

Sucrose gap recording revealed the TRH had a depolarising potency comparable to that of glutamate (Fig. 1*a*). Its action differed from that of glutamate in a number of ways. It was of considerably longer duration (Fig. 1*a*), considerably more variable from preparation to preparation, often showed tachyphylaxis, and the peak of the dose response curve was only 3.7 ± 1.4 mV (s.d.), whereas high concentrations of glutamate resulted in depolarisations of **10** to 20 mV. It is possible that the rapid breakdown of the TRH molecule contributes to the small size of the response, although the addition of a peptidase inhibitor, bacitracin[4], (5×10^{-5} M) (Calbiochem) or lowering the temperature did not increase the size of the response. Blockage of synaptic transmission either with magnesium ions (10 mM) or tetrodotoxin (5×10^{-6} M) reduced the size of the TRH response by 10–40%, suggesting that there are both direct and indirect components to the depolarisations. Equimolar concentrations of pyroglutamate, histidine, or prolinamide, the amino acid constituents of the TRH molecule, either had no effect or an inhibitory action, indicating that the depolarisation cannot be explained by the activation of amino acid receptors by one of these amino acids following breakdown of TRH to its constituent amino acids. Furthermore, the 1-methyl-histidine-TRH analogue was devoid of activity, while the 3-methyl-histidine-TRH analogue was approximately 10 times more potent than TRH.

Intracellular recording from motoneurones ($n=43$) confirmed that TRH had a depolarising action and that maximum depolarising concentrations resulted in a low level depolarisation (5.6 mV ± 3.23 s.d., $n=43$) which never

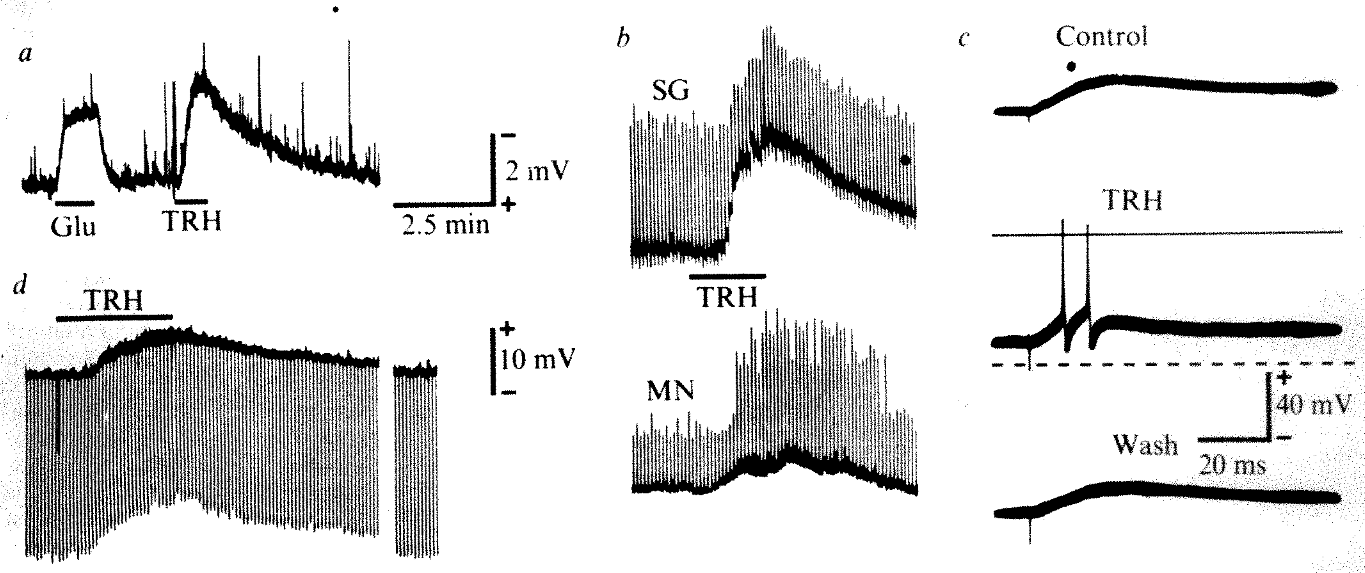

Fig. 1 Depolarising action of TRH on frog motoneurones. *a*, depolarisation of motoneurones to L-glutamate (10^{-4} M) and TRH (10^{-4} M) recorded with sucrose gap from a ventral root. The rapid depolarising deflections result from spontaneous synaptic input to the motoneurones. In *b*, subthreshold e.p.s.p.s which appear as upward deflections reach threshold during TRH depolarisation (5×10^{-4} M). Top trace (SG) is sucrose gap pen record from ventral root and bottom record (MN) is the intracellular response simultaneously recorded from a motoneurone (resting potential, -77 mV). Dorsal root was stimulated every 5.5 s. Spikes during depolarisation in lower record are attenuated in the pen record. *c*, Film records showing e.p.s.p. and spikes from another motoneurone (resting potential, -75 mV). In *d*, constant current hyperpolarising pulses were applied through the microelectrode every 5.5 s which appear as downward deflections (resting membrane potential, -85 mV). The membrane resistance fell from 3.1 MΩ to 2.8 MΩ during TRH. The gap in the record is 5 min. The time calibration in *a* also applies to the other chart records. The voltage calibration in *a* (negativity up) also applies to the sucrose gap record in *b*. The voltage calibration in *d* (negativity down) also applies to the intracellular record in *b*.

reached firing threshold in uninjured cells. This depolarisation did, however, have an excitatory action, raising subthreshold excitatory postsynaptic potentials (e.p.s.p.s) and depolarising current pulses above threshold. This is shown for e.p.s.p.s in Fig. 1*b* (bottom record) and *c*, from two different motoneurones. To analyse the mechanism underlying the depolarisation, membrane resistance was monitored by passing constant current hyperpolarising pulses. In 11 out of 21 motoneurones examined a small decrease in resistance occurred during the depolarisation (Fig. 1*d*). The inability to detect a resistance change in some cells is probably due to the small size of the depolarisation and to limited current carrying capacity of the microelectrodes. The resistance change seen with TRH was of a similar magnitude to that produced by an equivalent depolarisation by glutamate, suggesting that TRH increases conductance to sodium ions.

In summary, the present results indicate that TRH produces in frog motoneurones a long duration depolarisation of small magnitude which is accompanied by an increase in membrane conductance and excitability. Very similar results have been observed with the peptide substance P (refs 15, 16 and unpublished observations). Such actions suggest that TRH and substance P act in a background manner to facilitate transmission through more powerful synaptic pathways subserving reflex activity. The excitatory action of TRH on motoneurones is in contrast to its inhibitory action on supraspinal neurones[8–11] raising the possibility that it may act as an excitatory transmitter on one cell type and as an inhibitory transmitter on another. In this regard the excitatory action described in the present report is perhaps more consistent with the behavioural effects of TRH which are generally of a stimulant nature[3–7], and with its ability to release thyrotropin from the anterior pituitary, the latter being associated with an excitation of anterior pituitary cells[17]. Interestingly the structural requirements for the behavioural effects[18], releasing activity[13], and membrane excitatory actions reported here are all quite similar. The direct neuronal effects of TRH strengthen the concept that TRH may play a neurotransmitter role in addition to and possibly unrelated to its well established neuroendocrine role, although similar receptors are involved in both functions.

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Saturation of a retinal cone mechanism

IN discussions of the several differences between the rods and the cones of the human retina it is usually stated that rods saturate and cones do not. When measurements are made of the intensity (ΔI) of an incremental flash that can just be detected on a steady background field of intensity I, the cones normally continue to obey Weber's law ($\Delta I/I$ = constant) at very high intensities when nearly all their pigment has been bleached away; but when only a very small fraction of rhodopsin, the rod pigment, has been bleached the incremental threshold for rods begins to rise more rapidly than is predicted by Weber's law. This saturation of the rod signal can be observed when, by careful choice of wavelengths and by exploitation of the directional selectivity of the cones, the rod response is followed to high intensities in normal observers[1]; or when measurements are made on rod monochromats, whose retinae completely, or almost completely, lack cone receptors[2-4]. Alpern, Rushton and Torii[5] have suggested that it is bleaching that prevents saturation in the cones, for the receptor signal depends on the number of quanta actually absorbed and the latter quantity will tend to a limit as more and more pigment is bleached by steady background fields of increasing intensity. Cone mechanisms do saturate when intense, brief flashes are delivered to the unbleached retina[5,6]. We show here that the blue-sensitive receptors, which are normally thought to be cones, can be saturated by steady fields. In this respect they resemble the rods rather than the red- or green-sensitive cones.

Since the green cone mechanism is almost as sensitive to blue light as is the blue mechanism[7], it was necessary to choose experimental conditions carefully in order to follow the isolated response of the blue mechanism to high intensities. The test flash was violet (435 nm); and was large (1° of visual angle) and long (200 ms) in order to take advantage of the spatial and temporal integrative properties of the blue mechanism[8,9]. It was delivered to the fovea, fixation being guided by an array of four small fixation lights arranged in a diamond. A bright yellow (575-nm) 'auxiliary field'[10], which subtended 6.5°, served to maintain the light adaptation of the long- and middle-wavelength mechanisms; it produced a retinal illuminance of 5.48 log troland (equivalent to 0.17 log erg s^{-1} degree^{-2}) and remained present throughout the experiment. The primary adapting field (445 nm) was of variable intensity and was congruent with the auxiliary field. The arrangement of the stimuli is shown in Fig. 1*d*. The experiment was under computer control and a random double-staircase procedure[11] was used to measure the threshold for detecting the violet test flash at each of an increasing series of intensities of the 445-nm adapting field. Four minutes of light adaptation preceded measurements at each new level of the 445-nm field. Other details of procedure, apparatus and calibration were as described elsewhere[12].

Results are shown in Fig. 1*a* and *b*. The broken line has a slope of 1 and represents Weber's law. Clear evidence of the onset of saturation is seen in the rapidly rising increment-threshold function. Blue–violet fields that cause saturation are not very bright: the increment-threshold function exceeds a slope of 1 when the 445-nm field has a value of ~ 100 trolands, although, of course, the photopic troland is not an appropriate measure of light absorbed by the short-wavelength receptors.

At the highest field intensities the function suddenly flattens, and we suggest that here the green cones have taken over the detection of the violet flashes. To test the latter proposal directly we measured the spectral sensitivity of the eye when it was adapted to the most intense 445-nm field, the auxiliary field still being present: different test wavelengths were used in random order and measurements were always made between 4 and 10 min after the onset of adaptation, during which period the threshold is known to be stable for the observer used (J.D.M.). Measurements were made at two wavelengths in each session and at least 3 h elapsed between sessions. The results (Fig. 1*c*) are well fitted by π_4, Stiles's green-sensitive cone mechanism[7]. They could in principle be fitted by the red mechanism, π_5, but definitely not by π_3, the blue mechanism (which is plotted in Fig. 2*c*).

The reader will ask why saturation of the blue receptors has not previously been recorded, either by Stiles or by the many

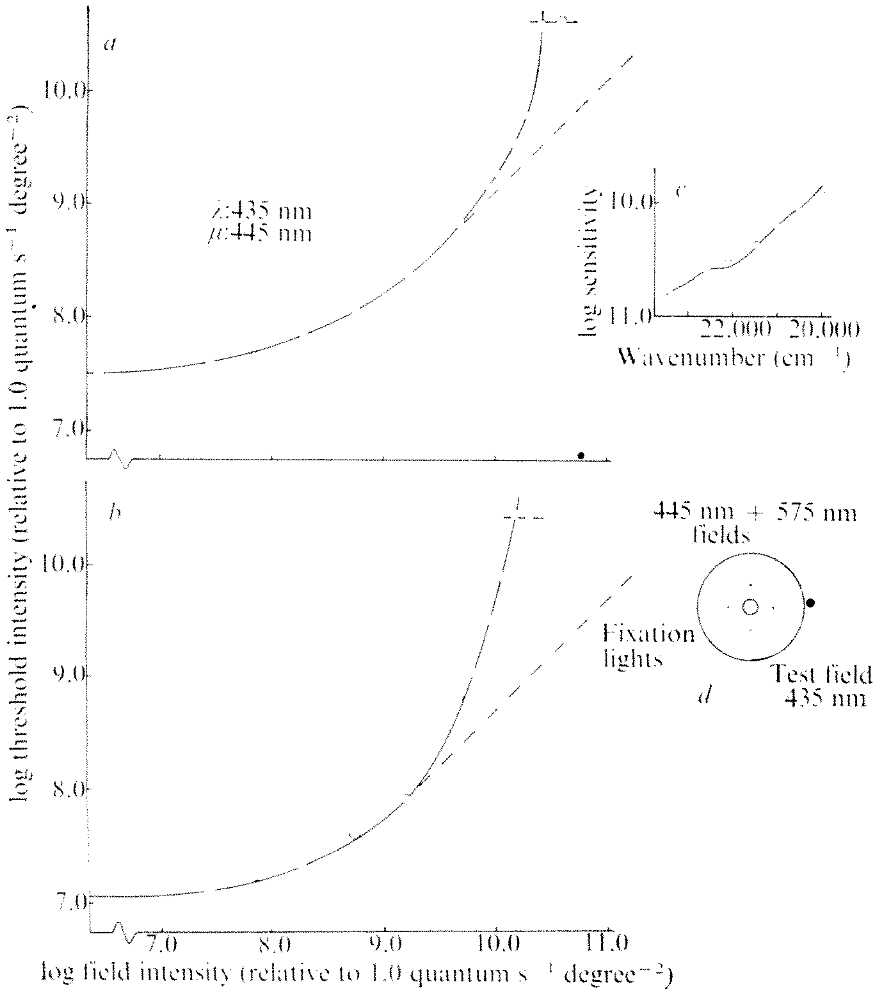

Fig. 1 *a*, *b*, Incremental threshold (Δ*I*) for the 435-nm test flash as a function of the 445-nm primary adapting field. The left-most point represents the threshold on the yellow auxiliary field alone. Observers: J.D.M. (*a*), P.G.P. (*b*). *c*, Spectral sensitivity of the eye after 4 min adaptation to the most intense 445-nm field (10.57 log quanta s^{-1} degree^{-2}). The ordinate is log sensitivity, that is, log reciprocal threshold (relative to 1.0 quantum s^{-1} degree^{-2}). The solid line represents the spectral sensitivity of Stiles's green mechanism, π_4, and has been arbitrarily displaced vertically. *d*, Arrangement of the stimuli.

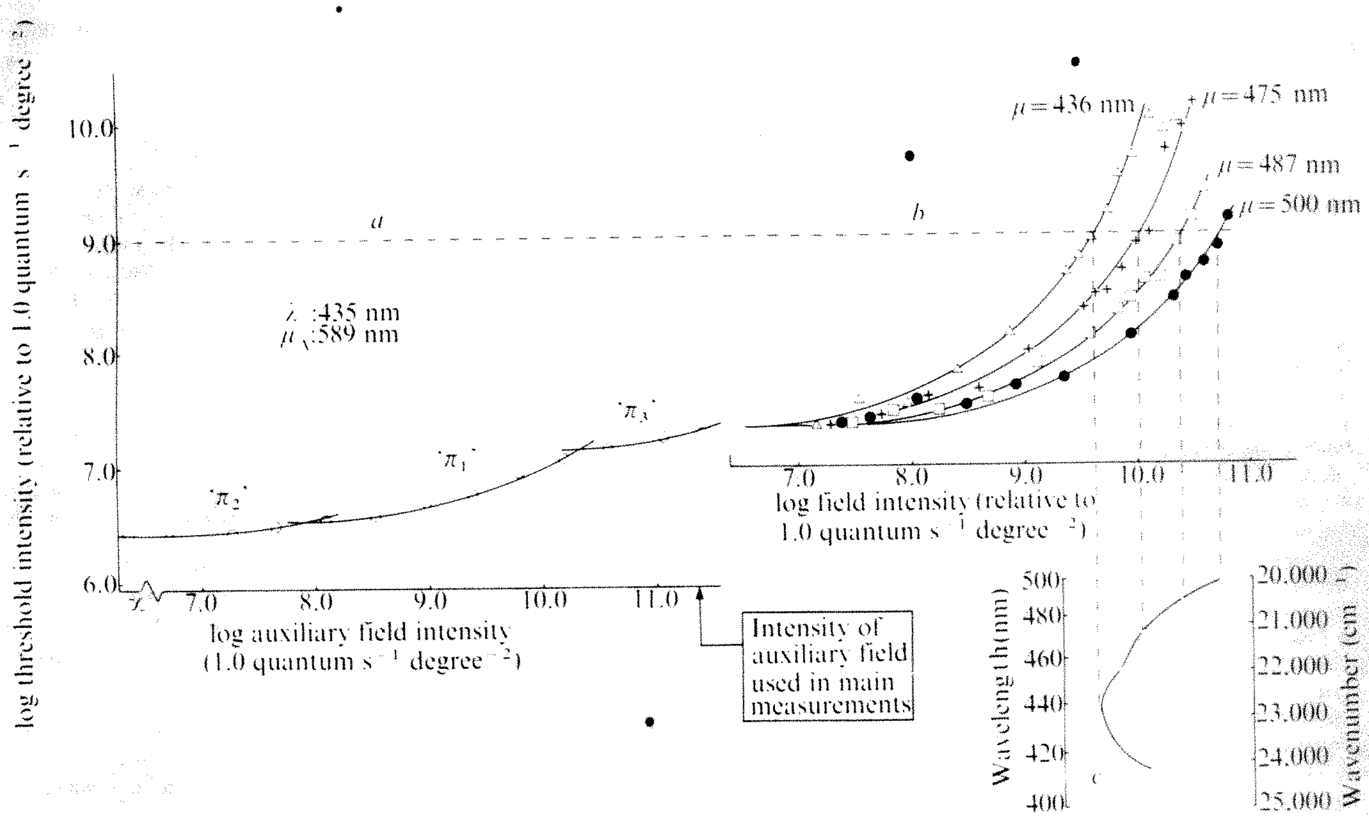

Fig. 2 *a*, Incremental threshold for a 435-nm test flash as a function of the intensity of the orange field alone. The curves fitted to the data points in each case correspond to Stiles's function $\zeta(x)$, taken from Table 7.5, ref. 7; and the relative position on the ordinate of the π_1 and π_2 branches is derived from the tabulated sensitivities of these mechanisms (Table 7.6, ref. 7). The reader should place less weight on the π_2 branch, which has been arbitrarily placed. Observer: J.D.M. *b*, Incremental threshold as a function of the intensity of the main adapting field (μ). The parameter is the wavelength of the main adapting field (μ). The auxiliary field was present throughout. The solid curve is empirical and has been displaced laterally to give the best fit to each set of data. Note that quanta absorbed from the auxiliary field are not represented by the abscissa of this plot. (The theory of the auxiliary field is discussed by Stiles in ref. 10). *c*, Field intensities that produce criterion saturation plotted against wave number. The solid line represents the field sensitivity of Stiles's high-intensity blue mechanism, π_3.

others who have measured increment thresholds using his two-colour procedure. A simple explanation is that many experimenters have not had available the necessary intensities of short wavelength light. But since Stiles is usually cited in support of the claim that cones are never saturated by steady fields, it is interesting and reassuring to note that Fig. 11 of the paper[10] published by Stiles in 1953, which represents data collected in conditions similar to those used here, does show an increment threshold function with a final slope that is greater than 1. Stiles used a 555-nm auxiliary field of −2.15 log erg s⁻¹ degree⁻², which was less intense than that of the present experiment: thus the intrusion of π_4 would occur earlier and would mask the most steeply rising part of the function of Fig. 1*a* and *b*. Norren and Padmos[19] have presented electroretinographic evidence that the dynamic range of the blue mechanism is limited.

What causes Weber's law to fail for the blue receptors? There is good evidence that at lower intensities the blue mechanism is subject to inhibition from a long-wavelength mechanism and that Stiles's three blue mechanisms, π_1, π_2, and π_3, correspond to the same receptors in different states of inhibition (for discussion see refs 12 and 13). Does the saturation seen in Fig. 1 arise directly from quanta absorbed by the short-wavelength receptors or is the blue mechanism being inhibited by a mechanism with a different spectral sensitivity? If the former is the case, we should find the lights of different wavelength that produce a given degree of saturation are lights that from other evidence would be thought to produce equal absorption in the blue receptors; but if the alternative hypothesis were correct, then the results of varying the wavelength of the adapting field ought to reflect the spectral sensitivity of the putative inhibitor. These expectations were the basis for a second experiment, in which the wavelength of the primary field was varied.

The conditions for the second experiment were similar to those of the first, but the wavelength of the auxiliary field (μ_A) was lengthened from 575 to 589 nm, in order better to reveal Stiles's π_3 mechanism, and its brightness was reduced to 5.2 log troland (−0.03 log erg s⁻¹ degree⁻²). The left-hand panel of Fig. 2 shows the effect of the orange auxiliary field alone on detection of the violet test flash. The three shallow branches correspond to Stile's mechanisms π_1, π_2, and π_3. The final level of the orange field (marked with an arrow) was that used as the auxiliary field during the main measurements and it is seen to raise the threshold by about one $\log_{10}$ unit.

Primary adapting fields of varying wavelengths were then added to the orange auxiliary field, a single experimental session being devoted to each wavelength. Results for four adapting wavelengths are shown in Fig. 2*c*; the results for other wavelengths are not shown to avoid congestion on the plot, but they are very similar in form.

Each set of data can be fitted well by a curve of fixed form that obeys Stiles's displacement rule[14], being displaced along the abscissa without vertical shift or other distortion. Figure 2 shows graphically how from these data can be derived the spectral sensitivity of the mechanism underlying saturation. We take as an arbitrary criterion that field intensity at which the slope of the increment-threshold function is 2. (Since the curve is of fixed shape, any criterion will give the same answer.) For each adapting wavelength this value is projected downwards and plotted against wavelength together with the corresponding points for sets of data not shown. The spectral sensitivity function that results (open triangles, Fig. 2*c*) is extremely well fitted by Stiles's curve for π_3, which is taken from ref. 7 (Table 7.6) and which can on several grounds be regarded as a good estimate of the spectral sensitivity of the blue receptors. That the fit is good must follow from the fact that the increment threshold functions of Fig. 2*b* have a fixed shape, for Stiles's measurements of the field sensitivity of π_3 were made in similar conditions but with a lower criterion.

Basic to this derivation is the assumption that Rushton[15] has called the principle of univariance: the output of a receptor depends only on the number, and not on the wavelength, of the quanta caught. As we vary wavelength, all that will change is the proportion of incident quanta that are absorbed. If the data of Fig. 2*b* could be plotted in terms of quanta actually absorbed then all the curves would coincide; but the abscissa is in fact the number of quanta delivered to the cornea and thus each curve is displaced laterally by a distance equal to the log of the ratio of quanta incident to quanta absorbed. It is these displacements that give us the spectral sensitivity of that which causes the saturation.

We conclude that the blue mechanism saturates when the blue-sensitive receptors are themselves absorbing a fixed number of quanta from a field. The present experiment offers no evidence for inhibition.

The saturation of the blue receptors at relatively low intensities may account for several long-standing problems of colour vision. It may, for example, explain why violet lights show a Bezold–Brücke hue shift towards longer wavelengths, coming to appear pale blue as their intensity is increased[16,17].

We have some evidence that if adaptation is maintained at saturating levels for more than 15 min, a recovery of sensitivity occurs: this further anomaly, which recalls physiological findings for rods in the skate[18], is under investigation.

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ATP-dependent calcium storage in presynaptic nerve terminals

CALCIUM ions, which enter presynaptic nerve terminals during depolarisation, trigger transmitter release from the terminals of both peripheral[1] and central[2] neurones. The subsequent disposition of the entering Ca is not entirely settled. Although this Ca must eventually be extruded in order for the neurones to return to the steady state, some of it may be temporarily retained and sequestered in intraterminal organelles. We now present evidence for an intraterminal, ATP-dependent Ca storage system which may help to regulate internal ionised Ca concentrations. This latent Ca sequestering system has been revealed by lysis of pinched-off presynaptic nerve terminals ("synaptosomes") in hypotonic media, followed by measurements of ATP-dependent Ca uptake from media containing mitochondrial inhibitors. These observations provide an alternative to the suggestion[3,4] that intraterminal mitochondria may be the primary Ca storage sites.

Synaptosomes were prepared from homogenates of rat brain cerebral cortex by the procedure of Hajós[5]. The synaptosomes (in 30–40 ml of 0.8 M sucrose), were diluted[6] to about 200 ml with an ice-cold buffered physiological salt solution (145 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 2.4 mM NaH_2PO_4 and 20 mM HEPES [*N*-2-hydroxyethyl piperazine-*N*′-2-ethanesulphonic acid] buffered to *p*H 7.65 at 20 °C with Tris [hydroxymethyl] aminomethane base); the protein concentration, after dilution, was 0.11–0.15 mg ml^{-1}. Aliquots of the saline-diluted suspensions were centrifuged at 15,000*g* for 5 min at 5 °C. The supernatant solutions were discarded, and the synaptosome pellets were resuspended and used for the ^{45}Ca uptake studies described below (see figure legends for details). In each experiment, several representative pellets were assayed for protein by the Lowry method[7].

Following incubation in ^{45}Ca-labelled fluids, aliquots (containing 0.2–0.3 mg protein) of the suspensions were pipetted on to 2.5 cm diameter, 0.3 μm pore diameter Millipore filters (Millipore, Bedford, Massachusetts). The fluid was filtered by suction, and the filters were washed three times with 4 ml aliquots of ice-cold physiological salt solution (see above) containing 1.2 mM $CaCl_2$. The ^{45}Ca content of the material on the filters, and of samples of the ^{45}Ca incubation solutions, was determined by standard liquid scintillation spectrometry.

There is considerable evidence that synaptosomes reseal and exhibit functional properties similar to those of intact nerve terminals when incubated in physiological salines[2-8].

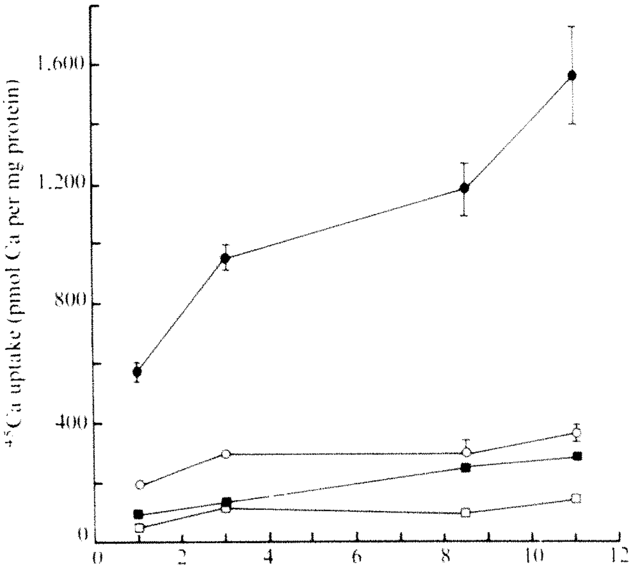

Fig. 1 Time course of ^{45}Ca uptake by intact and disrupted synaptosomes in the presence and absence of ATP. The "intact" synaptosomes (□, ■) were incubated in 2 ml of the standard physiological salt solution (see text) containing 10 μM $CaCl_2$ labelled with ^{45}Ca; the solution also contained 0.1 mM DNP, 0.1 mM Na azide and 0.7 μg ml^{-1} oligomycin. Other ('disrupted') synaptosomes (, ●) were resuspended and incubated in 2 ml of a solution similar in composition except for the omission of all the NaCl; the sudden exposure to the markedly hypotonic media presumably induced lysis[8]. When 2 mM ATP was present (●, ■), the $MgCl_2$ concentration in the incubation fluid was increased to 3 mM; open symbols refer to tissue incubated in ATP-free solutions. Incubations were carried out at 30 C; the protein concentration in the suspensions was 0.41 mg ml^{-1}. Each symbol represents the mean of three determinations; error bars are shown where the standard errors extend beyond the borders of the symbols.

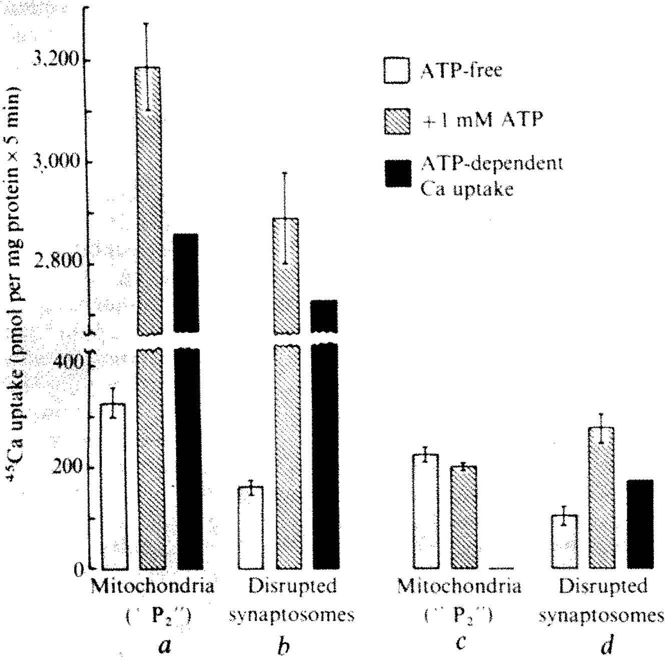

Fig. 2 Comparison of ^{45}Ca uptake by mitochondria and disrupted synaptosomes. The "crude mitochondrial pellet" ("P$_2$", ref. 10) was suspended in about 30 ml of ice-cold 0.32 M sucrose. Half of the suspension was used to prepare a synaptosome-enriched fraction by the method of Hajós[5]. The remainder was immediately diluted with about 100 ml of ice-cold standard physiological saline; isotonic solutions were used in order to avoid lysis of the nerve terminals present in this fraction (see Fig. 1). Aliquots (6 *ml*) were pipetted into centrifuge tubes and the particulate material was pelleted by centrifugation at 15,000*g* for 5 min at 5 °C. The supernatant solutions were discarded, and the pellets (1.39 mg protein) were suspended in 1.0 ml of physiological saline with all the NaCl replaced by KCl (*a*,*c*); the solution pipetted into some tubes (*a*, *c*) also contained DNP, NaN$_3$ and oligomycin (to give final concentrations of 0.1 mM, 0.1 mM and 0.7 μg ml^{-1}, respectively). After 1 min of incubation at 30 °C, 1 ml of similar K-rich saline containing 10 μM Ca labelled with ^{45}Ca was added; the solution added to some tubes (see graph) also contained ATP (final concentration = 1 mM). Following a 5-min incubation at 30 °C, aliquots of the suspensions were filtered and treated as described in the text. The synaptosomes obtained by gradient centrifugation[5] of the sucrose-suspended "P$_2$", were diluted with saline and centrifuged (see text). The pellets (0.93 mg protein) were suspended in 1 ml of "lysis solution" (standard physiological saline with all the NaCl omitted) (*b*, *d*); the solution used to suspend some of the pellets also contained the three mitochondrial poisons (*d*). After 1 min of incubation at 30 °C, 1 ml of buffered saline containing 290 mM KCl (and no NaCl) was added to each suspension; this solution contained 10 μM CaCl$_2$ labelled with ^{45}Ca and, where appropriate (see graph) ATP (final concentration = 1 mM). Following a 5-min incubation at 30 °C aliquots of the suspensions were filtered and treated as described in the text. Each bar represents the mean of three determinations; error bars indicate ± s.e.m.

Figure 1 shows that when the external Ca concentration is about 10 μM, ATP has little effect on Ca uptake by intact synaptosomes incubated in the physiological salt solution with an osmolarity of about 320 mosM. If, however, the synaptosomes are disrupted by exposure to markedly hypotonic media[5], a new Ca uptake process appears—one which is greatly enhanced by ATP (Fig. 1). The fact that it is observed in lysed, but not in "intact", preparations suggests that the uptake mechanism is associated with intraterminal organelles. Following lysis, re-introduction of a 320 mosM solution does not abolish the ATP-stimulated Ca uptake (see Figs 2–4). This ability to withstand large changes in osmotic pressure is reminiscent of fragmented sarcoplasmic reticulum, which can be isolated in 30 mM KHCO$_3$ and then returned to an approximately 300 mosM solution for Ca uptake studies[9].

Participation of mitochondria in this ATP-stimulated Ca uptake can be ruled out, because all incubation solutions contained 0.1 mM 2,4-dinitrophenol (DNP) and 0.1 mM sodium azide (two mitochondrial uncouplers), and 0.7 μg ml^{-1} oligomycin (an inhibitor of mitochondrial ATP-promoted Ca uptake); this combination of poisons blocked virtually all (99±1%; $n=4$) of the Ca uptake in control

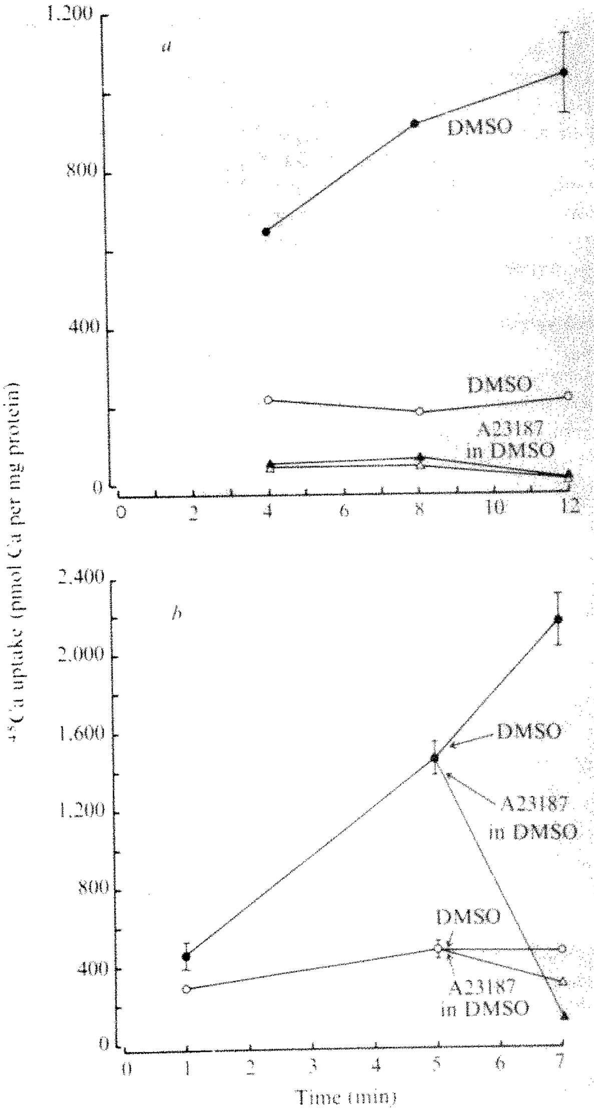

Fig. 3 Effect of ionophore, A23187, on ^{45}Ca uptake by disrupted synaptosomes. Data in *a* and *b* are from different synaptosome preparations. In both experiments, the synaptosomes were disrupted by pre-incubating them for 1 min in 1 ml of standard physiological salt solution with the NaCl omitted. Incubation fluid (1 ml) was then added to provide a (final) composition similar to that of the standard solution, but with all of the NaCl (145 mM) replaced by KCl. In addition, the incubation fluid contained sufficient ^{45}Ca-labelled CaCl$_2$ to give a final concentration of 10 μM in Ca; when 2 mM ATP was present (●, ▲), the MgCl$_2$ concentration was increased to 3 mM. All lysis and incubation fluids contained 0.1 mM DNP, 0.1 mM Na azide and 0.7 μg ml^{-1} oligomycin. All incubations were carried out at 30 °C. Each symbol represents the mean of three determinations; error bars are shown where the standard errors ranged beyond the borders of the symbols. *a*, ^{45}Ca-labelled incubation fluids containing dimethylsulphoxide (DMSO; final concentration = 4.2 μl ml^{-1}) without (○, ●) or with A23187 (△, ▲; final concentration = 10 μM), were added at zero time; the DMSO was used as a solvent for the ionophore stock solution. The protein concentration in the suspensions was 0.51 mg ml^{-1}. *b*, The ^{45}Ca-containing solutions were added at zero time. As indicated on the graph, DMSO (final concentration = 2.5 μl ml^{-1}), without or with A23187 (final concentration = 10 μM), was added immediately after the 5 min samples were drawn. The protein concentration in the suspensions was 0.49 mg ml^{-1}.

experiments on rat liver and rat brain mitochondria. Figure 2 shows data from a representative experiment in which we directly compared Ca uptake by disrupted synaptosomes and by brain mitochondria (crude mitochondrial fraction "P_2" of Gray and Whittaker[10]). Note that, in the presence of the mitochondrial poisons, ATP significantly increased Ca uptake by the disrupted synaptosomes (Fig. 2*d*) but had no effect on Ca uptake by the P_2 fraction (Fig. 2*c*).

Several observations indicate that the ATP stimulates Ca uptake rather than superficial (surface) binding: (1) The extra ^{45}Ca on the filters is not displaced by the non-radioactive Ca (1.2 mM) in the wash solutions (all experiments). (2) There is no stimulation of Ca uptake by ATP, even after 7 min of incubation, if the incubation is carried out at 3 °C rather than at 30 °C (data not shown). (3) The divalent ion-specific ionophore, A23187, prevents the ATP stimulation of Ca uptake (Fig. 3*a*) and releases previously accumulated Ca (Fig. 3*b*). These data are consistent with the hypothesis that ATP induces "uphill" (that is, against an electrochemical gradient) transport of Ca into vesicular compartments[11]. (4) The ATP-stimulated Ca uptake at long incubation times is enhanced when oxalate is present in the incubation medium (Fig. 4). If oxalate is passively distributed across the membranes of the Ca-accumulating particles, as appears to be the case in fragmented sarcoplasmic reticulum[12], the data in Fig. 4 imply that ATP promotes net transport of Ca into the vesicles so that the intravesicular Ca concentration rises and the calcium oxalate solubility product is exceeded. Atomic absorption measurements of total Ca (unpublished) indicate that ATP (in the presence of mitochondrial poisons) promotes net Ca accumulation by the particulate material in the disrupted synaptosome preparations; in the presence of ATP, oxalate increases the net uptake. (5) The effect of ATP on Ca uptake is greatly reduced in the absence of Mg (data not shown), perhaps indicating that a Mg-dependent ATPase may be involved in Ca sequestration[9,13].

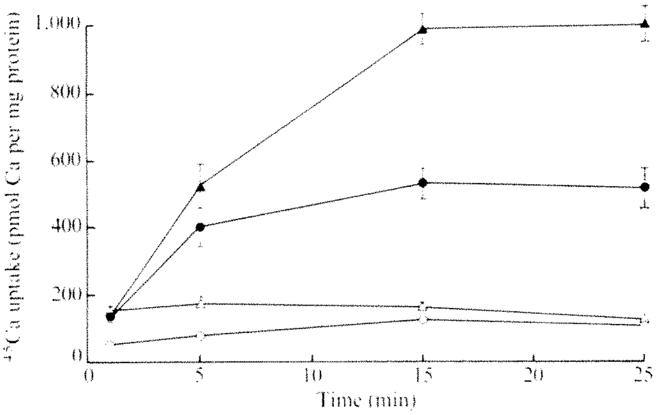

Fig. 4 Effect of oxalate on the time course of ^{45}Ca uptake by disrupted synaptosomes. Synaptosomes were suspended in 1.0 ml of lysis fluid (standard salt solution with NaCl omitted); after 1 min (time zero on the graph) ^{45}Ca-containing solutions (1.0 ml) were added to give a final Ca concentration of 5 μM. The final composition of the incubation solutions was similar to the standard salt solution except that all of the NaCl was replaced either by 145 mM KCl (oxalate-free solutions; , ●) or by 5 mM K oxalate plus 137.5 mM KCl (, ▲); all solutions contained 0.1 mM DNP, 0.1 mM Na azide and 0.7 μg ml^{-1} oligomycin. When 2 mM ATP was present (●, ▲), the $MgCl_2$ concentration was raised to 3 mM. Incubations were carried out at 30 °C; the protein concentration in the suspensions was 0.49 mg ml^{-1}. Each symbol is the mean of three determinations; error bars are shown where the standard errors range beyond the borders of the symbols.

In summary, these experiments suggest that there are vesicular bodies, other than mitochondria, localised within presynaptic nerve terminals, which can accumulate Ca in the presence of ATP and Mg. The properties of this transport system resemble those of fragmented sarcoplasmic reticulum[9,11–14]. Similar non-mitochondrial ATP-dependent Ca sequestration has also been observed in whole brain microsomes[15,16]; however this activity could not be assigned to the synaptic region because of the heterogeneity of the preparations.

Preliminary kinetic data indicate that the ATP-dependent Ca uptake by the disrupted synaptosome preparations has a half-saturation for Ca of less than 1 μM; this sequestration mechanism may therefore operate at physiological intracellular Ca concentrations. By comparison, mitochondria apparently have a lower affinity for ionised Ca: in the presence of physiological K and Mg concentrations, half-saturation for Ca uptake by these organelles may be in excess of 10 to 20 μM Ca (ref. 17). Thus, as an alternative to the hypothesis that mitochondria may be the main intraterminal Ca storage sites[2–4], our data are consistent with the idea that the mitochondria may help to buffer intraterminal Ca primarily by supplying ATP to the transport and storage system described above. This system may therefore play an important role in facilitation and post-tetanic potentiation because these phenomena seem to be the consequence of Ca retention by presynaptic nerve terminals[18,19].

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Chemical properties of human Ia antigens

THE human major histocompatibility complex encompasses at least four well-defined loci. Those called HLA-A, -B, and -C control the expression of the classical transplantation antigens (ref. 1). The fourth locus, HLA-D, controls determinants which regulate the ability to stimulate in mixed leukocyte cultures (MLR) and seems at least partly to correspond to the I-region of the murine H-2 complex. A great deal of work has been done on the immunogenetics of the HLA-D locus (reviewed in ref. 2) but despite the fact that alloantibodies against HLA-D determinants are available[3,4] the molecular nature of the antigens is largely unknown. Such studies have been hampered by the fact that HLA-D determinants only seem to be expressed on a small proportion of the peripheral blood lymphocytes (the B cells)[4] but Fu *et al.*[5] have reported data which suggest that B-cell-specific alloantigen is expressed on leukaemic cells. We have therefore isolated a cell membrane glycoprotein complex, controlled by the HLA–D locus, from leukaemic cells of a patient with chronic lymphatic leukaemia. The characteristics of the protein complex strongly suggest that it represents the human equivalent of the murine Ia antigens.

Patient P.S. suffering from chronic lymphatic leukaemia was investigated with respect to the HLA phenotype. Routine tissue typing with a panel of well-defined HLA alloantisera established the phenotype pattern to A1, A2, B8, Bw40, Cw3. The phenotypic pattern of the HLA–D locus was assessed with the MLR test with use of a panel of homozygous typing cells[6]. A clearly positive response for Dw3 was obtained but no other HLA–D specificity could unambigously be assigned to the patient. Additional testing with HLA–D alloantiserum-induced cytotoxicity[7] on the leukaemic cells strongly suggested that the HLA–D phenotype was Dw3 and Dw4.

An alloantiserum obtained from a multiparous woman strongly inhibited the MLR response when the stimulating cells were of the Dw3 phenotype. No effect of the antiserum was noted when lymphocytes of a Dw3 homozygous donor were used as the responder cells and the stimulator cells were of another HLA–D type. The antiserum was as effective in impeding the MLR response even after absorption on platelets of the relevant HLA type, but after absorption, the antiserum was only cytotoxic for B lymphocytes of the Dw3 donor origin. Another alloantiserum which blocked the MLR response stimulated by cells expressing Dw4, was rendered specific for the HLA–D locus determinants by the same procedure.

Leukocytes of patient P.S. were obtained by leukocytopheresis and the lymphocytes were isolated by Ficoll–Isopaque gradient centrifugation[8]. Cell-surface proteins of the isolated cells were labelled by lactoperoxidase-catalysed iodination and Triton X-100-solubilised molecules reactive with the alloantisera directed against Dw3 and Dw4 determinants were precipitated and analysed by SDS-polyacrylamide gel electrophoresis according to a procedure detailed elsewhere[9] (Fig. 1). It is evident that both antisera precipitated two types of polypeptide chains with apparent molecular weights 28,000 and 34,000. No molecules of the HLA-type, that is with apparent molecular weights 12,000 and 45,000, were observed suggesting that the absorption of the antisera had been successful. The specificity of the antisera was also ascertained by the fact that normal human serum did not react with either of the two types of polypeptide chains (Fig. 1*c*). Moreover, absorption of the alloantiserum against Dw3 determinants on Dw3-expressing homozygous cells completely abolished its reactivity with the two polypeptide chains. On the other hand, absorption of this alloantiserum on Dw4-expressing homozygous B lymphocytes did not diminish its reactivity with the two polypeptide chains from Dw3-expressing cells.

In other experiments leukaemic cells of patient P.S. were cultured in the presence of ^{3}H-leucine and ^{3}H-fucose, respectively[9]. Radiolabelled molecules reactive with alloantisera against Dw3 and Dw4 determinants were isolated and analysed by SDS–polyacrylamide gel electrophoresis. With either of the radioactive labels the two polypeptide chain pattern was evident. Consequently, both polypeptide chains contain carbohydrate moieties.

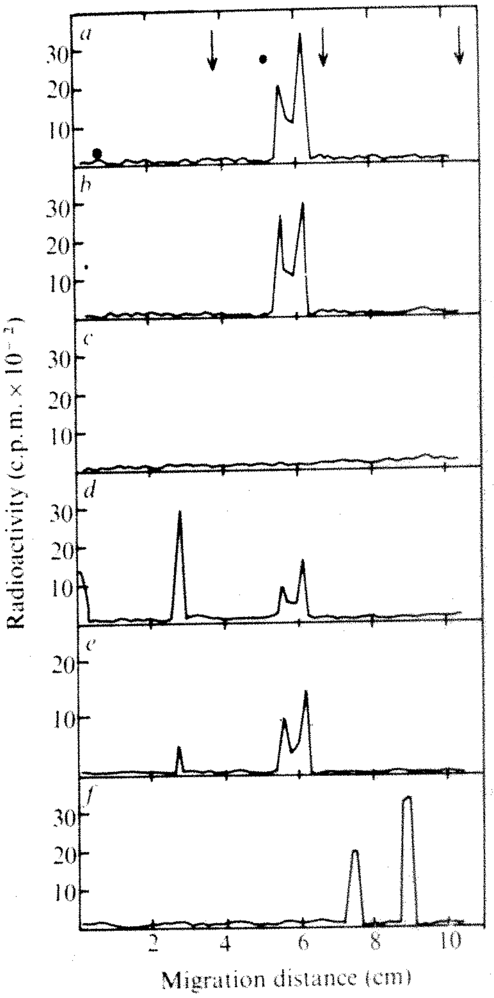

Fig. 1 SDS–polyacrylamide gel electrophoresis under reducing conditions of Triton X-100-solubilised, ^{125}I-labelled membrane antigens reactive with alloantisera directed against HLA-Dw3 (*a*) and HLA-Dw4 (*b*) determinants. No molecules reacted with a normal human serum (*c*). ^{125}I-labelled membrane molecules, crosslinked with TDH, were precipitated by a sandwich procedure using an alloantiserum directed against Dw3 determinants and analysed by SDS–polyacrylamide gel electrophoresis (*d*). The component with the apparent molecular weight 60,000 was extracted from the gel, treated with 15 mM $NaIO_4$ to cleave the crosslinker, and resubjected to SDS–polyacrylamide gel electrophoresis (*e*). The same material as in *a* was analysed by SDS polyacrylamide gel electrophoresis in the absence of reducing agents (*f*). The arrows denote the migration positions (from left to right) of marker heavy and light chains of IgG and the marker dye bromphenol blue. (*a–e*). The markers in (*f*) were immunoglobulin light chains (molecular weight 23,000, migration distance 10·2 cm), ovalbumin (molecular weight 43,000, migration distance 6.0 cm) and bovine serum albumin (molecular weight 68,000, migration distance 3.0 cm).

Since the SDS–polyacrylamide gel electrophoretic analyses were performed in reducing conditions, we looked to see whether the two types of polypeptide chains might be associated by disulphide bonds. In the absence of reduction the two polypeptide chains were separated and, thus, no covalent interaction was apparent (Fig. 1*f*). The two polypeptide chain pattern may result, however, from the existence of non-covalent interactions between the chains in physiological conditions. Alternatively, the alloantisera may react with two independent molecules. To resolve this question Triton X-100-solubilised, ^{125}I-labelled membrane proteins were crosslinked with the cleavable crosslinker tartryl-di(glycylhydrazine) (TDH)[10] and reacted with the alloantiserum against Dw3. Three types of molecules were observed after SDS–polyacrylamide gel electrophoresis (Fig. 1*d*). In addition to the two usually encountered polypeptide chains a new component with the apparent molecular weight 60,000 was evident. The latter was extracted from the gel, the crosslinker cleaved by treatment with $NaIO_4$ (ref. 10), and the material resubjected to SDS–polyacrylamide gel electrophoresis. The result (Fig. 1e) demonstrates that the 60,000-dalton component most probably comprised one polypeptide chain of each type, held together in the denaturing conditions by the crosslinker.

An intriguing observation is that the smaller of the two HLA-D chains always contains more radioactivity than the larger chain regardless of whether external or internal labelling has been used. This result may suggest that the small chain contains more of the labelled residues (leucine, tyrosine, and fucose) than the large chain. Alternatively, the small chain may have a greater turnover than the large chain and consequently may incorporate more of the radioactive precursors. In addition, the small chain could conceivably be more exposed on the membrane surface than the large chain which would account for the results obtained with lactoperoxidase-catalysed iodination (compare ref. 13). Further studies are needed to resolve these possibilities.

Further evidence for a firm non-covalent interaction between the two types of polypeptide chain was obtained from molecular weight determinations in non-denaturing conditions. A large scale isolation of material reactive with the anti-Dw3 alloantiserum was performed. From crude cell membranes of about 200 g of leukaemic cells (expressing the HLA-D specificities Dw3 and Dw4) sodium deoxycholate-solubilised molecules were purified by successive frationation steps involving affinity chromatography on lectin columns and gel chromatography[11]. Of the material in the most highly purified fraction about three-quarters reacted with the HLA-D alloantisera whereas the remainder of the material comprised unrelated molecules. Part of the HLA-D reactive material in this fraction was labelled with ^{125}I and subjected to analytical gel chromatography and sucrose density gradient centrifugation in deoxycholate. The distribution of molecules expressing the allotypic determinants was assessed by indirect immunoprecipitation. The molecules reactive with the alloantisera were eluted earlier than marker HLA molecules and they also sedimented faster than the HLA antigens. The Dw3-expressing molecules were analysed by SDS polyacrylamide gel electrophoresis and the peak fractions always displayed patterns similar to those shown in Fig. 1*a* and *b*. From the values of the Stokes' radius (44 Å) and the sedimentation constant (4.4S) a molecular weight for Dw3 and Dw4-expressing molecules of about 83,000 could be calculated[12]. This molecular weight represents the sum of the weights of the individual polypeptide chains and the bound detergent. Since each one of the two types of polypeptide chains seems to bind an appreciable amount of detergent, but displays much lower values on sedimentation velocity analyses than the intact component (L.S.-T. unpublished observations) it seems likely that the 83,000 dalton complex comprises the two polypeptide chains in a 1 : 1 ratio and about 20,000 daltons of bound detergent.

The present data do not formally exclude the possibility that the glycoprotein complex is composed of two identical polypeptide chains which are very susceptible to proteolysis. No molecules comprising chains of a single type have ever been observed however, and in ^{3}H-leucine labelling experiments the label occurs simultaneously in the two chains. This strongly suggests that the two types of chain are not involved in a precursor–product relationship.

The data reported above suggest that the isolated components may be the human equivalents of the murine Ia antigens (see ref. 13). To investigate this problem in some detail we have raised a rabbit antiserum against the protein complex. Fab fragments of these antisera abolished the lymphocytotoxic action of all HLA-D alloantisera tested and blocked the MLR response when bound to stimulator but not to responder cells in the MLR (L.K., unpublished observations and Ken Welsh, personal communication). These antisera were specific for the HLA-D determinants of normal peripheral blood lymphocytes as revealed by indirect immunoprecipitations and SDS-polyacrylamide gel electrophoresis (Fig. 2), and after solubilised membrane molecules had been reacted with the heteroantisera no molecules remained in the supernatant which bound to the relevant HLA-D alloantisera. One of the antisera (from Ken Welsh) reacted weakly but significantly with ^{3}H-leucine-labelled B10.S splenocyte membrane molecules and, as can be seen in Fig. 2, the polypeptide chain pattern on SDS-polyacrylamide gel electrophoresis is that of typical murine Ia antigens. The heteroantisera did not cross react with HLA antigens derived from the A, B and C loci. Neither did heteroantisera directed against the A, B and C locus gene products[1] react with the glycoprotein complex. This suggests that there is no apparent immunological similarity between the molecules coded for by the D locus and those coded for by the A, B and C loci.

The present data in conjunction with previous reports[15-17] strongly suggest that the HLA-D locus controls the expression of polypeptide chains which are the equivalents of the murine Ia antigens. Like the latter molecules the HLA-D-derived antigens are composed of two types of glycoprotein chains exhibiting immunological cross reactivity with murine Ia antigens which in non-denaturing

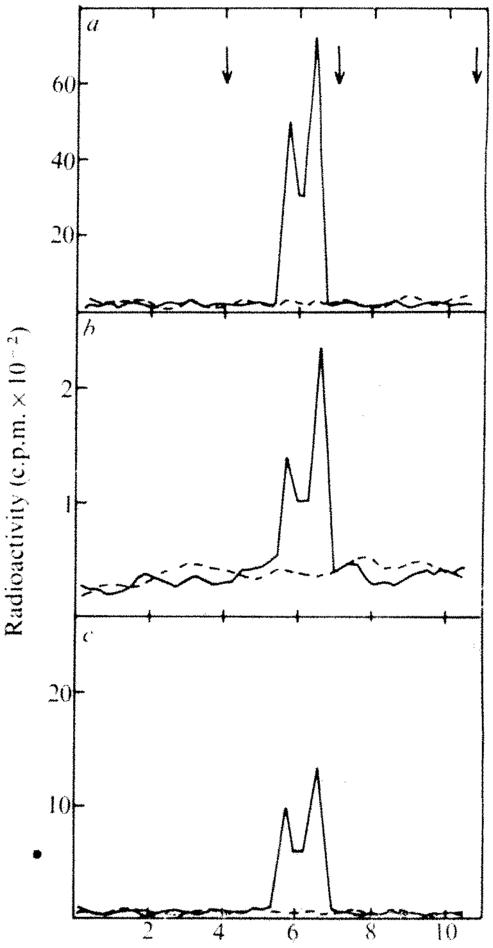

Fig. 2 SDS-polyacrylamide gel electrophoresis of Triton X-100 solubilised, ^{125}I-labelled membrane antigens of lymphocytes from normal peripheral blood reactive with a rabbit antiserum directed against the 83,000-dalton glycoprotein complex (*a*). In (*b*) the same antiserum was reacted with ^{3}H-leucine-labelled B10.S splenocyte macromolecules, and in (*c*) the rabbit antiserum was substituted for an A.TL anti-A.TH serum (anti-Ia^s). In *a*, *b*, and *c* the broken curves represent molecules reactive with normal rabbit serum (*a* and *b*) and normal mouse serum (*c*). The arrows denote the migration positions (from left to right) of marker heavy and light chains of IgG and the marker dye bromphenol blue.

conditions form a stable complex comprising one chain of each type. The protein–protein association seems to involve non-covalent interactions only. Available information does not demonstrate if both polypeptide chains express alloantigenic determinants. It is therefore possible that only one of the chains, as for the HLA antigens[14], is controlled by the HLA region. Studies in progress are aimed at elucidating this question.

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Recognition of a naturally occurring idiotype by autologous T cells

BONE-MARROW-derived (B) lymphocytes belonging to the same clone bear antigen receptors which share a specific marker (idiotype) with antibodies secreted by their progeny[1]. According to the network theory of immunity[2], immunocompetent B cells recognise each other through a network of idiotype anti-idiotype interactions. This theory implies the existence of B and T cells specific for autologous idiotypes. To facilitate detection of such cells it is advantageous to study an immune response of restricted heterogeneity. The immune response of BALB/c mice to phosphorylcholine (PC) is idiotypically homogeneous: the anti-PC antibodies produced bear almost exclusively the idiotype characteristic of the BALB/c PC-binding myeloma protein TEPC-15 (T15) (ref. 3). T15 is a member of a family of PC-binding myeloma proteins of BALB/c origin, which bear distinct idiotypes[4]. Idiotypes representative of these other proteins are not, however, expressed by BALB/c on immunisation with PC. It has recently been reported that helper T cells from BALB/c mice can discriminate among idiotypes expressed on several of these anti-PC myeloma proteins. These authors did not, however, demonstrate helper T cells specific for the T15 idiotype[5]. This failure was attributed to a state of tolerance induced and maintained by the high levels of immunoglobulin bearing the T15 idiotype in conventional BALB/c mice. In this report we demonstrate the existence of T cells reactive to an autologous idiotype and we discuss the possible regulatory role of these T cells in the immune response to PC.

To generate idiotype-specific T cells, conventional and neonatally suppressed BALB/c mice were immunised with T15. Neonatal suppression was accomplished by injecting 0.1 ml of A/J anti-T15 serum at birth as previously described[6]. Mice received a total of 400 μg of protein in a series of four injections over a period of 2 weeks. The first injection was given in complete adjuvant in the foot pads, the second was given in incomplete adjuvant, intraperitoneally (i.p.). The final two injections were given in saline i.p. At least four weeks after the last injection mice were sacrificed and T cells were purified from both spleen and pooled lymph nodes using the method of nylon wool filtration[7]. Dinitrophenyl (DNP)-specific B cells were obtained from BALB/c mice immunised with 100 μg of alum-precipitated DNP conjugated keyhole limpet haemocyanin (DNP_9-KLH) and 10^9 *Bordetella pertussis* organisms. These mice were rested for at least 6 weeks before sacrifice, when their spleen cells were treated with anti-Thy 1.2 serum and complement.

The T15 primed T cell populations were assayed for helper cell activity in a hapten-carrier adoptive transfer system with DNP-primed B cells and DNP-conjugated T15 (DNP_6-T15) as antigen. To demonstrate the specificity of the T15 primed T cells, helper activity was assessed in the same transfer system with DNP-conjugated McPC603 (DNP_6-M603) as antigen. M603 is another myloma protein of BALB/c origin with binding activity for phosphorylcholine and differs from T15 only in its idiotypic specificity[8,9]. Conjugation of either T15 or M603 with DNP did not alter the idiotype determinants which characterise these two myelomas (results not shown). Therefore, these idiotypes should be available as carrier-specific determinants.

DNP-primed B cells were transferred with T15-primed T cells into conventional recipients irradiated with 600 r. Each recipient received 50 μg of aqueous DNP_6-T15 along with the cells. The animals were sacrificed 8 d later and their spleens assayed for indirect anti-DNP plaque-forming cells (PFC) using the Cunningham and Szenberg[10] modification of the haemolytic plaque technique[11]. DNP-conjugated sheep erythrocytes prepared by the method of Inman *et al.*[12] were used as indicator cells.

Table 1 shows that when 3×10^6 DNP-primed B cells were transferred with 10×10^6 T15-primed splenic T cells obtained from suppressed animals, the resulting response was 2,340 indirect anti-DNP PFC per spleen. This response is 16-fold higher than the background response of 142 anti-DNP PFC calculated by summing the responses obtained on transfer of the same cell populations separately. Lymph node T cells from the same T15-primed suppressed animals did not provide any detectable helper activity above background. In addition, neither splenic nor lymph node T cells obtained from T15-primed conventional donors provided as efficient helper activity as splenic T cells obtained from T15-primed suppressed donors; although transfer of either of the non-suppressed T cell populations with DNP-primed B cells did result in an anti-DNP PFC response 6-fold higher than background (Table 1).

Since conventional BALB/c mice have high levels of circulating immunoglobulin (Ig) bearing the T15 idiotype[8], these molecules might compete with DNP_6-T15 and inhibit the helper activity of T15 specific T cells. To optimise the expression of the T15 specific helper cells we used irradiated (600 r) neonatally suppressed[6] BALB/c mice as recipients. These animals contained less than 0.68 μg ml^{-1} of circulating Ig bearing T15 idiotype compared with conventional BALB/c mice which contain between 40–80 μg ml^{-1}.

Table 1 Demonstration of idiotype specific helper T cells

DNP-KLH† primed B	Number of cells transferred (×10⁶)* Suppressed T15 primed‡ spleen T	lymph node T	Conventional T15 primed§ spleen T	lymph node T	Indirect anti-DNP PFC/spleen (×10²)¶
3	—	—	—	—	0.49 (0.30–0.78)
–	10	—	—	—	0.93 (0.66–1.32)
–	—	10	—	—	0.68 (0.30–1.51)
–	—	—	10	—	< 0.4
–	—	—	—	10	< 0.2
3	10	—	—	—	23.4 (13.8–39.8)
3	—	10	—	—	2.9 (2.0–4.3)
3	—	—	10	—	5.4 (3.4–8.6)
3	—	—	—	10	5.0 (2.9–8.9)

*Mixtures of cells were transferred into 600 r irradiated conventional BALB/c mice intravenously. Animals received 50 μg of aqueous DNP$_6$–T15 simultaneously with transferred cells. Animals were sacrificed 8 d after cell transfer.

†DNP-KLH primed spleen was treated with anti-Thy 1.2 serum and complement resulting in a population containing 94% immunoglobulin bearing cells and 2% T cells as assessed by immunofluorescence.

‡Suppressed BALB/c donors were given 0.1 ml of A/J anti-T15 serum intraperitoneally at birth. At 2 months of age suppressed animals were immunised with two injections of 100 μg of TEPC15 per week for 2 weeks. T cells were isolated from spleen and pooled lymph nodes using nylon wool fractionation. The purified splenic T population contained 93% T cells and 1.8% immunoglobulin-bearing cells and the lymph node T population contained 97% T cells and 1.4% immunoglobulin-bearing cells (as assessed by immunofluorescence).

§Conventional BALB/c mice were immunised with TECP15 as the suppressed animals. Splenic T cells and pooled lymph node T cells were purified using nylon wool. The resulting spleen T-cell population contained 90% T cells and 1% immunoglobulin-bearing cells and the lymph node T-cell population contained 95% T cells and 0.6% immunoglobulin-bearing cells (as assessed by immunofluorescence).

¶Geometric mean of the responses of 4 or 5 animals per group. Numbers in parentheses represent one s.e. about the mean. The direct PFC response in all groups was less than 5% of the total response. Indirect PFC were developed using a polyvalent rabbit anti-mouse immunoglobulin serum.

When 2×10^6 or 10×10^6 splenic T cells obtained from T15 primed suppressed mice were transferred along with 3×10^6 DNP primed splenic B cells into suppressed recipients, the resulting anti-DNP PFC responses were 50-fold and 20-fold higher than background, respectively (Table 2). These T cells are specific for the T15 idiotype since transfer of the same cell population with DNP$_6$-M603 as antigen did not result in an anti-DNP response above background (Table 2).

Table 2 Enhanced expression of idiotype specific helper activity in idiotype suppressed recipients*

DNP-KLH‡ primed B	Number of cells transferred† (×10⁶) Suppressed T15 Primed§ Spleen T	Antigen	Indirect anti-DNP PFC per spleen (×10²)¶
3	—	DNP$_6$–T15	3.1 (2.4–4.1)
–	20	DNP$_6$–T15	4.3 (2.6–6.9)
3	20	DNP$_6$–T15	376.0 (301–469)
3	10	DNP$_6$–T15	151.0 (118–193)
3	10	DNP$_6$–M603	8.5 (5.0–14.5)

*All recipients were injected with 0.1 ml of A/J anti-T15 serum intraperitoneally at birth. Recipients were 3 months of age at time of transfer and contained < 0.68 μg ml^{-1} of Ig sharing idiotypic determinants with T15.

†Mixtures of cells were transferred into 600 r irradiated idiotype-suppressed BALB/c mice with 50 μg of aqueous antigen. Animals were sacrificed 8 d after transfer.

‡DNP-KLH primed spleen cells were treated with anti-Thy 1.2 serum and complement resulting in a population containing 93% immunoglobulin bearing cells and 0.7% T cells as assessed by immunofluorescence.

§Spleen T cells were isolated from idiotype-suppressed TEPC15-primed BALB/c mice; see legend to Table 1. The resulting population contained 93% T cells and 1.2% immunoglobulin-bearing cells as assessed by immunofluorescence.

¶Geometric mean of the responses of 3 or 4 animals per group. Numbers in parentheses represent 1 s.e. about the mean. The direct anti-DNP PFC response in all groups was less than 5% of the total response. Indirect PFC were developed using a polyvalent rabbit anti-mouse immunoglobulin serum.

The greater efficiency with which T15-specific helper activity is generated in neonatally suppressed compared with conventional BALB/c mice is perhaps related to the fact that suppressed mice have 100-fold lower concentrations of circulating Ig bearing the T15 idiotype[6]. The high levels of T15 idiotype in conventional BALB/c might partially mask the T15-specific T-cell clones. Consistent with this hypothesis is the finding that T15-specific B cells are also more readily induced in suppressed than conventional BALB/c mice on immunisation with T15 (H.C., A.A. and M.H.J., in preparation). The ability of conventional mice to produce anti-T15 antibodies demonstrates that the T15-specific precursor cells are present but simply not expressed to the same extent as in suppressed mice. Similarly, T15-specific T cells can be induced in conventional BALB/c but not as readily as in suppressed mice.

Splenic T cells isolated from suppressed unprimed BALB/c and transferred along with DNP-primed B cells into suppressed irradiated recipients, failed to give an anti-DNP response significantly above background (data not shown). Thus, if mature T15-specific helper T cells exist in unprimed suppressed BALB/c, there are too few to be detected and/or antigen (T15)-dependent proliferation and maturation of T15-specific precursor T cells might be required for generation of helper activity.

Idiotype-specific T and B cells might play a part in regulation of the immune response to PC. We have recently demonstrated that BALB/c mice can produce antibodies specific for the T15 idiotype. These autologous anti-idiotypic antibodies modulate the idiotypic pattern of the immune response to PC *in situ*, that is, the proportion of antibodies produced which bear the T15 idiotype is reduced compared with normal controls (H.C., A.A. and M.H.J., in preparation). The production of these antibodies is T dependent since 'B mice' (adult thymectomised, lethally irradiated and foetal liver reconstituted) fail to produce anti-T15 antibodies on immunisation with T15 (M.H.J., unpublished).

Although we have shown that idiotype-specific T cells can function as helpers in the production of anti-DNP antibodies, these T cells might also collaborate with B cells of the same specificity in the production of autologous anti-T15 antibodies. Alternatively, analogous to the studies of Eichmann[13], T15-specific T cells could function as suppressor cells and directly regulate the expression of PC-specific B cells expressing receptors bearing the T15 idiotype. We are currently investigating the modes of action of T15-specific T cells in an attempt to define their possible role in regulation of the anti-PC response.

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Altered expression of human monocyte Fc receptors in malignant disease

SEVERAL kinds of cells involved in immunity can bind antibody or immune complexes through surface receptors which interact with the Fc portion of IgG (Fc receptors). The expression of Fc receptors on mononuclear phagocytes (monocytes/macrophages) can undergo changes in response to various stimuli both *in vitro*[1] and *in vivo*[1-3] and such changes may play a regulatory role in the induction of an immune response and in immunological effector mechanisms. Here I show that the expression of Fc receptors on circulating human monocytes is substantially increased in individuals with solid malignant tumours. This change seems not to be a normal characteristic of infectious or inflammatory diseases of a non-malignant nature.

The experimental group consisted largely of individuals with various bronchogenic carcinomas (squamous cell, oat cell, adenocarcinoma) and included examples of carcinoma of the rectum, pharynx, oesophagus and breast. All were untreated and the majority were without signs of metastatic disease. Control subjects were healthy laboratory workers and were initially age and sex matched with patients until it became evident that significant age and sex differences were not present amongst normal donors. In the study of non-malignant diseases individuals with the most active disease were selected where possible, thus for example, all pulmonary tuberculosis patients had live bacilli in their sputum.

Lymphocytes and monocytes were isolated from 10 ml of heparinised peripheral blood by centrifugation over a Ficoll/triosil gradient. The cells were washed twice, adjusted to approximately 10^6 ml^{-1} in Hank's balanced salt solution (BSS) and 1-ml aliquots of this were placed in each chamber of dual tissue culture slides (Lab-tek products). After incubation at 37 °C for 90 min the glass adherent cells were washed three times with BSS. This procedure resulted in a monolayer consisting of monocytes and a proportion of quite firmly adherent lymphocytes. The former were easily distinguishable from lymphocytes because of the spreading which occurred following monocyte adherence. However, this morphological identification was confirmed by latex particle ingestion[4].

Fc receptor expression was characterised using an assay similar to that described for guinea-pig macrophages[1]. A panel of erythrocytes, bearing increasing amounts of specific antibody, was prepared by incubating a 1% suspension of ox erythrocytes with heat-inactivated rabbit anti-ox antiserum at increasing concentrations in phosphate-buffered saline. After 30 min at 20 °C the cells were washed once and resuspended in BSS at a concentration of 1%. One-millilitre aliquots of sensitised erythrocytes were added to each monolayer and allowed to settle for 1 h at 20 °C. The monolayer were then washed five times with BSS, fixed with 1% glutaraldehyde and stained with buffered Giemsa. Mounted preparations were examined and cells with five or more attached erythrocytes were scored as rosettes. The percentage of rosette-forming monocytes occurring at each increment of erythrocyte sensitisation in a monolayer from a normal donor was compared with that occurring simultaneously in a monolayer from a donor with malignant disease. In the study of non-malignant diseases the comparison between monocytes from

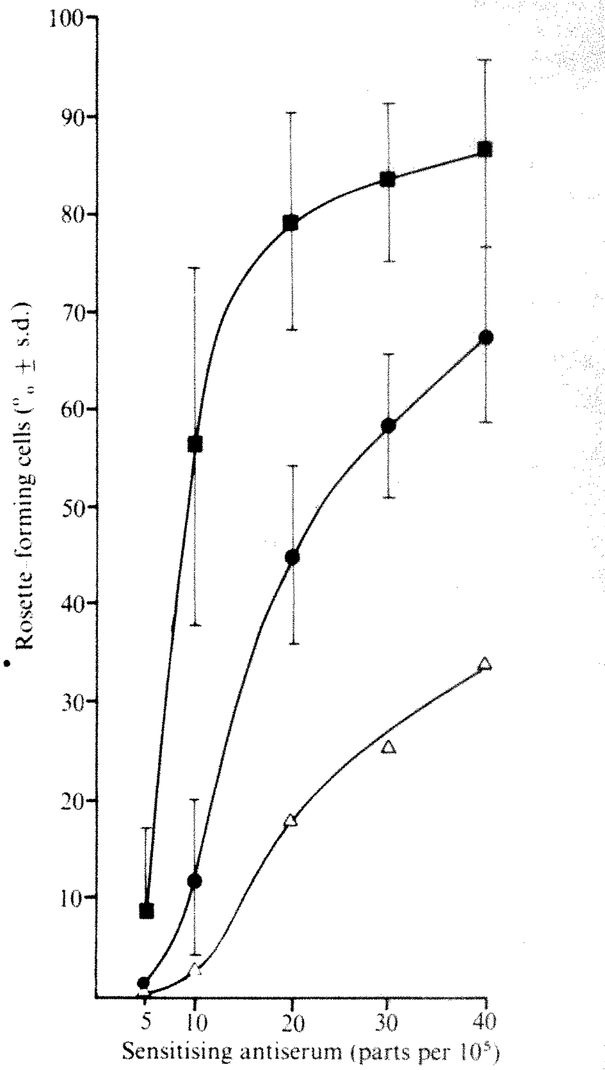

Fig. 1 The percentage of rosette forming monocytes as a function of the degree of erythrocyte sensitisation in fourteen individuals with malignancies (■), in seventeen normal individuals (●), and in one individual with malignant disease (lung cancer) and maturity-onset diabetes (△). Values are expressed as means ± one standard deviation.

Table 1 Occurrence of B lymphocytes, T lymphocytes and T lymphocytes expressing Fc receptors (FcR), as judged by rosette formation, in peripheral blood of individuals with malignant tumours

	Non-T lymphocytes expressing FcR (B lymphocytes and others) % of total	T lymphocytes % of total	T lymphocytes expressing FcR % of total
JC	36.0	64.0	2.5
TJ	35.0	65.0	5.0
MC	35.5	64.5	7.0
RH	39.0	61.0	6.0
SF	35.0	65.0	3.0

Lymphocytes isolated from 10 ml of heparinised peripheral blood on Ficoll/triosil gradients were assayed by the method of Haegert *et al.*[5]. Cells expressing FcR bind ox erythrocytes sensitised at an optimal concentration of antiserum (60 parts per 10^5) while T lymphocytes bind fluorescein-labelled sheep erythrocytes. Any T lymphocytes expressing FcR bind both indicator cells.

patients and from healthy donors was made using those two increments of sensitisation which had been established as optimal for detecting differences in receptor expression. Fc receptor expression by lymphocytes from a group of patients with untreated bronchogenic carcinoma was also characterised by a mixed rosette suspension assay in which T lymphocytes bound fluorescein-labelled sheep erythrocytes and B lymphocytes, and any other cells expressing Fc receptors, bound antibody-sensitised ox erythrocytes[5].

The dose–response curves in Fig. 1 show monocyte rosette formation as a function of the degree of erythrocyte sensitisation. At the second increment the proportion of monocytes binding immune complexes in patients with malignant disease is five times greater than normal. Fourteen of the fifteen patients tested showed a substantial increase in binding at all increments of sensitisation indicating a uniform increase in Fc receptor expresson rather than a change in the proportion of high avidity cells[1]. The one patient who did not exhibit elevated receptor expression showed instead a marked depression in comparison with normal (Fig. 1). This bronchogenic carcinoma patient also had maturity-onset diabetes mellitus and was receiving chlorpropamide at the time of sampling. Insulin inhibits the differentiation of macrophage Fc receptors *in vitro*[6] and a similar effect might explain this *in vivo* observation since higher than normal levels of insulin occur in maturity-onset diabetes[7]. It was not possible to demonstrate any increase in the expression of Fc receptors by T lymphocytes in patients with bronchogenic carcinoma. Very few T cells expressing Fc receptors were observed using the mixed rosette suspension assay of Haegert and associates[5] (Table 1).

The expression of Fc receptors by monocytes from individuals with non-malignant diseases is compared with that of normal donors in Table 2. Each datum is the largest difference observed in percentage rosette formation between a monolayer from the patient and a monolayer from a normal donor assayed simultaneously. Significant differences (greater than 20%) are boxed in for clarity and examples of malignant disease are included for comparison. Significant differences from normal do not seem to occur in the non-malignant diseases tested with the interesting exception of a proportion of patients with active pulmonary tuberculosis (three out of seven). Also, one patient with a surgical wound infection exhibits a significant difference while one patient with a breast lump later found to be malignant does not show significantly altered receptor expression.

Mononuclear phagocytes, in a variety of experimental systems, have been shown to exert a regulatory effect on the function of lymphocytes (reviewed by Nelson[8]). Macrophages seem to release both stimulatory and inhibitory factors which act on lymphocytes[9] while the macrophage surface may adsorb both cooperative[10] and suppressive[11] factors released by lymphocytes. In studies of the apparently complex relationship between macrophage and lymphocyte function one of the more consistent observations has been the suppression of T lymphocyte responses by activated macrophages[8]. In a study particularly pertinent to the present report Berlinger and associates showed that peripheral blood monocytes of patients with malignant tumours exert an inhibitory effect on the blastogenic response of the patients' lymphocytes in mixed

Table 2 Monocyte Fc receptor expression in non-malignant diseases

	No. of cases							
Pregnancy (week 32)	4	+12	+18	+7	−14			
Benign breast tumours	3	+3	+7	+12				
Rheumatoid arthritis	5	−7	+6	+14	+16	−17		
Gonorrhoea	6	−10	−3	−2	+8	−17	+3	
Pulmonary tuberculosis	7	−16	−11	+48*	+4	+53*	+37*	5
Chronic bronchitis	5	+4	−14	+4	−1	4		
Acute bronchitis	1	−1						
Bronchiectosis	1	−5						
Asthma (extrinsic)	2	−8	−17					
Viral meningitis	2	−11	−1					
Surgical wound infections	6	−11	+14	+42*	+1	−7	−10	
Dermatitis	4	−12	+6	−10	+13			
Squamous cell carcinoma of bronchus	(e.g.)	+66*						
Adenocarcinoma of bronchus	(e.g.)	+39*						
Carcinoma of rectum	(e.g.)	+63*						
Carcinoma of breast stage II	1	+35*						
Carcinoma of breast stage I	1	−9						

Monolayers were assayed using indicator cells sensitised with rabbit anti-ox antiserum at a concentration of 10 and 20 parts per 10^5. Each datum represents the largest difference in percentage rosette formation observed between a monolayer from a patient and from a normal donor assayed simultaneously. Significant differences are marked with an asterisk and examples of malignant disease are included for comparison.

leukocyte cultures[12]. The present study shows that altered expression of monocyte Fc receptors occurs in malignant disease but does not seem to occur readily in a variety of non-malignant diseases. This change in monocyte membrane characteristics may be an appropriate host response, facilitating macrophage-mediated cytotoxicity and the clearance of immune complexes, and tumour progression may occur because of lesions elsewhere in the host's defence system[13]. An alternative possibility is that the change in monocyte surface characteristics, or associated changes, occurring in malignant disease exert a net inhibitory effect on anti-tumour immunity. This might occur locally rather than systemically during the early growth of a tumour. The same macrophage-mediated inhibition of an immune response might also occur in chronic infections such as tuberculosis.

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Serological evidence for H–Y antigen in *Sxr*, XX sex-reversed phenotypic males

IT is generally considered that the Y chromosome is necessary for the development of the mammalian testis and the consequent male phenotype. Exceptions occasionally occur in man and other animals, where a male phenotype is associated with an apparently normal female karyotype. In some instances it has been possible to show that such "sex reversal" has an autosomal genetic basis. Although this might imply that the Y chromosome is not a prerequisite of maleness, it has not been excluded that a small male-determining region of the Y may have been translocated to an autosome without producing a recognisable karyotypic change[1] (reviewed in ref. 2). The best studied case of male development with an apparently female karyotype is provided by the *Sxr* mutant in the mouse. This autosomal dominant condition causes both XX and XO mice to develop as males, although germ cells are absent in *Sxr*,XX animals and spermatogenesis is impaired in *Sxr*,XO mice. *Sxr*,XY males have a slightly reduced testis size but are usually fertile. Cytological scrutiny of mitotic and meiotic chromosomes (including chromosome banding) has revealed no evidence for a Y–autosome translocation, although a small translocation undetectable by the methods used cannot be ruled out (reviewed in ref. 2). This situation can now be investigated using serological methods for detecting H–Y (histocompatibility-Y) antigen on the surface of mouse sperm[3] and male epidermal cells[4]. These techniques have provided further evidence that expression of this antigen is governed by a gene (or genes) on the Y chromosome even in the absence of the normal male phenotype[5]. Thus, serological demonstration of H–Y antigen offers a non-karyological criterion for the presence of a translocated portion of the Y in *Sxr*,XX males, because their cells should express H–Y if *Sxr* is a translocation involving the H–Y genetic determinant. We report here that *Sxr*,XX males express H–Y to a degree that may be the same or slightly lower than that of normal males, and that their *Sxr*,XY siblings seem to have near-normal levels of H–Y. The simplest explanation is that male-determining and H–Y-determining portions of the Y chromosome are present in the animals carrying *Sxr*.

The *Sxr* stocks were obtained from Harwell where they are maintained on a mixed C3H/HeH-101/H genetic background with tabby (*Ta*) being used as an X-chromosome

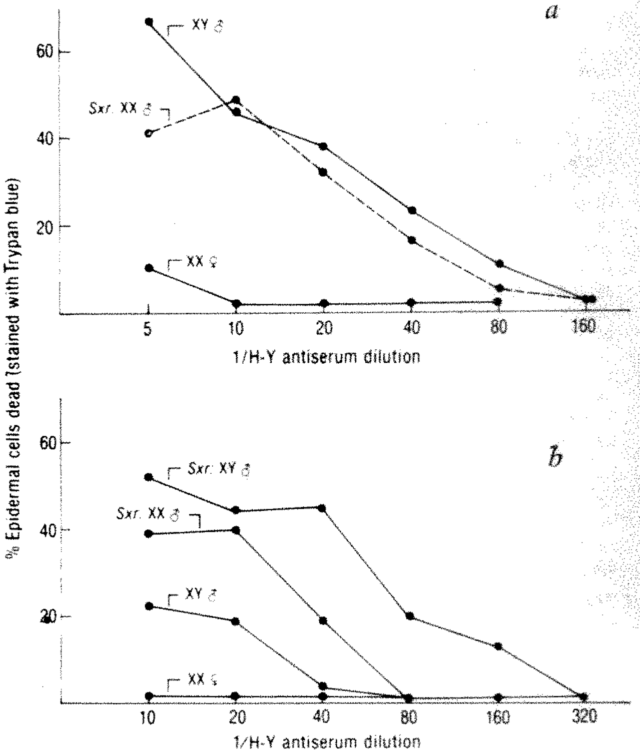

Fig. 1 Demonstration of H–Y antigen on dissociated epidermal cells of *Sxr*,XX and *Sxr*,XY mice by the complement-dependent cytotoxicity assay with anti-H–Y serum. *a*, The data in this figure are from a representative test with two animals in each group. The values shown are mean readings for the pairs of (C3H × 101)F$_1$ outcross stock controls and for the *Sxr*,XX individuals. Antiserum and compelment were the same as indicated in Fig. 1 of ref. 3. Percentage of epidermal cells dead (ordinate) was calculated as indicated in the legend to that figure. *b*, The data in this figure are from one of two sets where *Sxr*,XY males were compared with their sibling XY (non-carrier) males. The two classes of XY male siblings were distinguished on the basis of testis weight. Although this criterion does not provide absolute assurance of genotype, we identified putative *Sxr*,XY animals by testis weight in the extreme low range (0.069 g and 0.078 g) compared with XY males with testis weight in the extreme high range (0.102 g and 0.127 g). The siblings were selected from single litters and were therefore the same age at the time of the test.

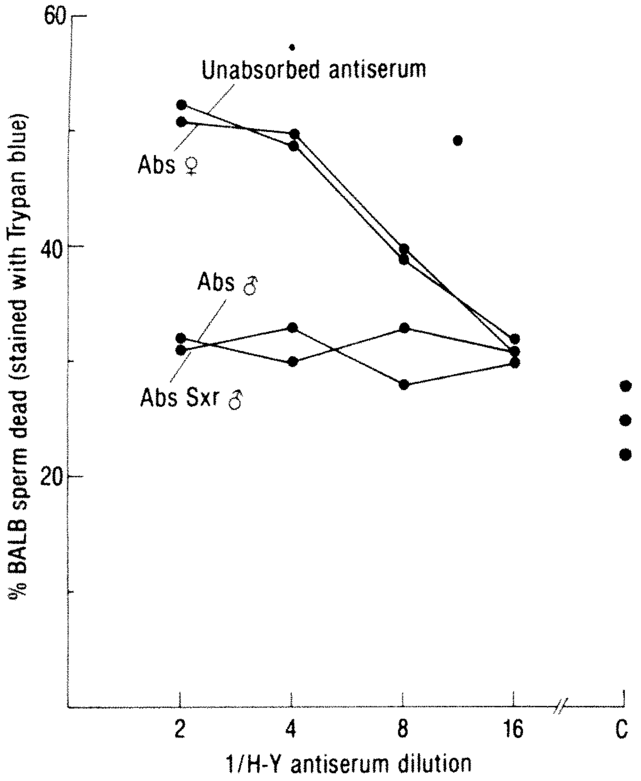

Fig. 2 Summary of cytotoxicity tests on mouse sperm with mouse H–Y antiserum after absorption with liver cells from normal females (B6), normal males (B6) and *Sxr*,XX males (see text). Abs, absorption with cells from the indicated sex. Each point represents an average value from three separate tests. C, control (complement included, antiserum omitted). Sperm suspensions were scored as coded samples. Tests were performed with BALB epididymal sperm according to Goldberg *et al.*[3], using serum raised in C57BL/6 (B6) female mice against B6 male spleen cells. Equal volumes (0.05 ml) of H–Y antiserum, sperm (5×10^6 per ml), and diluted rabbit serum (complement source) were incubated at 37 °C for 50 min. Trypan blue dye was added during the last 7 min of incubation to stain dead sperm. Absorption was accomplished by reacting 0.14 ml of H–Y antiserum (diluted 1/2) with an equal volume of packed liver cells. The cells were suspended in the antiserum for 30 min and then discarded. Absorption in the cytotoxicity test is manifested as a decrease in cytotoxic titre, indicating that H–Y antibody has been removed specifically from a particular aliquot by the cells used in the absorption procedure and that these cells must therefore contain H–Y antigen.

marker to distinguish the *Sxr*,XX male from the XY and *Sxr*,XY classes. Two types of mating are used: in one, *Sxr*,*Ta*/Y males are crossed with +/+ females (F_1 hybrid, C3H×101) and, in the other, *Sxr*,+Y males are crossed with *Ta*/+ females from a stock also maintained on the C3H/101 background. XY and *Sxr*,XY males are distinguished on the basis of testis weight and their ability to produce *Sxr*,XX progeny.

We tested *Sxr*,XX mice for the presence of H–Y antigen by the three methods described for *Tfm*/Y mice[5]. (1) In each of five separate experiments anti-H–Y serum gave a positive reaction in the cytotoxicity assay with dissociated epidermal cells from *Sxr*,XX males (Fig. 1*a*). In two of these experiments sibling XY males carrying *Sxr* (identifiable by small testes), and normal sibling XY males, were used as positive controls. In both experiments, one of which is shown in Fig. 1*b*, the anti-H–Y cytotoxicity reaction was stronger with *Sxr*,XY males than with normal XY males. This suggests the presence of excess H–Y antigen in *Sxr*,XY males (although this has not yet been corroborated by quantitative absorption of anti-H–Y serum) and thus might imply that *Sxr*,XY males have excess H–Y-determining genetic material. (2) The presence of H–Y antigen in *Sxr*,XX males was further indicated by a series of absorption tests; three of them, shown in Fig. 2, in which spleen, brain and liver cells of *Sxr*,XX males removed specific anti-H–Y reactivity from the H–Y antiserum. (3) C57BL/6 (B6) females grafted with *Sxr*,XX trunk skin were thereby specifically presensitised to give a second-set response when subsequently grafted with B6 male skin, indicating that the *Sxr*,XX skin graft was H–Y+ (Table 1).

These data support the interpretation that *Sxr* represents a cytologically undetected translocation between the Y chromosome and an autosome.

There are two possible mechanisms by which such a translocation may have arisen[1,2] (Fig. 3). If *Sxr* arose as a simple chromosome-type rearrangement between an autosome and the Y chromosome, the original male presumably possessed a normal complement of chromosome material, although the Y chromosome itself would be deficient and an autosome would carry a segment of Y chromosome. According to this scheme his *Sxr*,XX offspring would obtain a male-determining portion of the Y chromosome; his XY sons would have a deficient but nevertheless male-determining Y chromosome, and his *Sxr*,XY sons should have the same chromosome complement that was attributed to their father. But this hypothesis does not explain the defective testicular development seen in the original male or in his *Sxr*,XY sons[1] (reviewed in ref. 2). Therefore the more likely origin of this translocation involves a rearrangement of the chromatid-type in the father of the original male, in which case the original male could have retained a normal Y chromosome plus a duplication of a region of the Y chromosome involved in the translocation[1]. The defective testicular development of *Sxr*,XY males is understandable on the basis of this karyotype, since they would be partially equivalent to XYY males, which are known in the mouse to be subject to meiotic breakdown (reviewed in refs 2 and 6).

The interpretation of *Sxr* as a Y–autosome translocation may account for the different effect of *Sxr* on XX and XO genotypes. It has been proposed that the absence of germinal elements in XXY males arises because the presence of two active X chromosomes is incompatible with normal differentiation of male germ cells[7]. Early germ cell failure

Table 1 Second-set rejection of B6 male skin by B6 females previously grafted with *Sxr*,XX skin

First graft* (no. of B6 females grafted)	Rejection time (d) of B6 male skin grafted 2–3 months later							MST (confidence limits)
Sxr,XX (14)	10,	11,	12,	15,	15,	16,	16	14.5
	16,	16,	17,	18,	18,	19,	23	(16.3–12.9)
($C_3H \times 101$) F_1XY male controls (14)	12,	12,	12,	13,	13,	15,	15,	14.5
	15,	16,	17,	17,	17,	21,	22	(16.3–12.9)
($C_3H \times 101$) F_1XX female controls (8)	14,	16,	21,	23,	23,	26,	28	20.5
	30							(17.2–24.4)

Summary of data for two experiments involving four *Sxr*,XX graft donors and three donors for each of the two control groups; these donors also provided spleen cells and tail-epidermal cells for serological tests (Figs 1 and 2). Trunk skin was grafted according to Billingham and Medawar[8], using Band-Aids instead of plaster casts; rejection times were scored visually. Median survival time (MST) and confidence limits of the median were calculated by the method of Litchfield[9].

*See Fig. 1.

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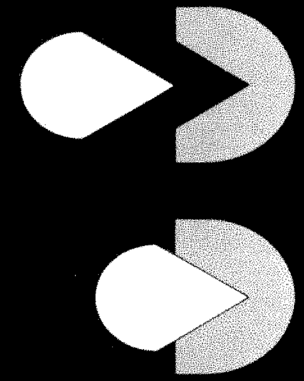

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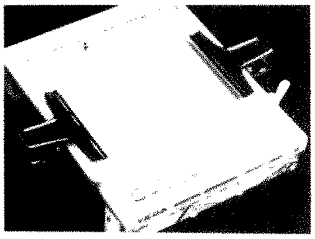

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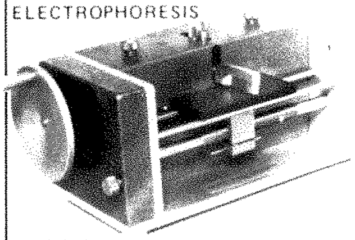
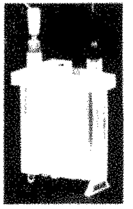
with channels
92.006/16

A COMPLETE SET OF COMPACT INSTRUMENTS FOR POLYACRYLAMIDE DISC GEL ELECTROPHORESIS



92.012 Gel Slicer Macrotome GTS



Preparative gel electrophoresis
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proteins and nucleic acids

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Extractor
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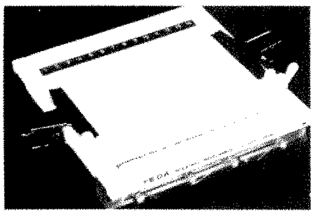
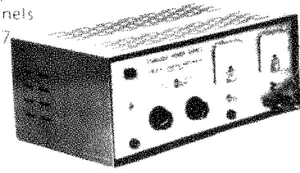
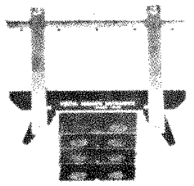


Flat plate electrophoresis
without channels
92.007/17



Stabilized Power
Supply
92.003

Separation
Chamber
92.009



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samples – precipitates and/or filtrates

Multiple Membrane Filter 92.051/2

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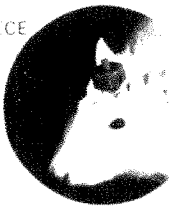
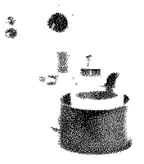
Infusion Cannula
92.103A

Injection Cannula
92.103B





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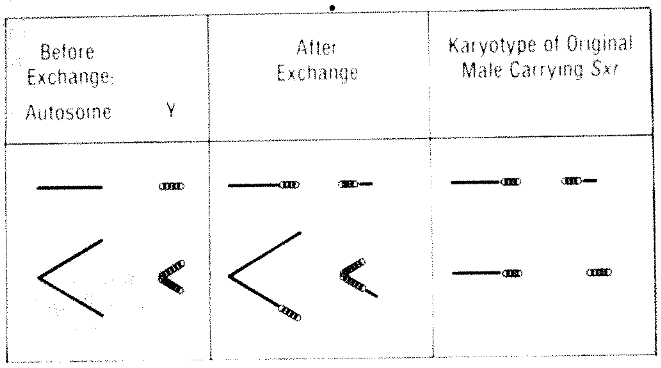

Fig. 3 Alternative hypotheses for translocational origin of *Sxr*. Upper, chromosome aberration; lower, chromatid aberration.

in *Sxr*,XX males and more extensive spermatogenesis in *Sxr*,XO males reinforces this argument. Furthermore, since spermiogenesis in *Sxr*,XO males is not completely normal, the translocated Y is presumably incomplete.

Although the data account for the male development of mice with an apparently female karyotype, different mechanisms must be responsible for the sex-reversed conditions found in goats and pigs (reviewed in refs 2 and 6). In these species the sex reversal is inherited as an autosomal recessive and this is not immediately understandable in terms of a Y–autosome translocation. Clearly, the H–Y status of these sex-reversed individuals requires investigation.

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Time and mode of fusion of human fibroblasts treated with polyethylene glycol (PEG)

SOMATIC cell fusion has become a powerful tool in cell biology and genetics[1–3]. Until recently the standard reagent for increasing the rate of fusion in cultures of animal cells was inactivated Sendai virus[4]. In 1974 polymers of ethylene glycol (polyethylene glycol, PEG), over a wide range of molecular weights, were shown to be very effective, non-toxic chemical 'fusogens' for higher plant protoplasts[5]. They were then applied to fuse hen erythrocytes[6] and these with yeast protoplasts[7]. Applied to mammalian, including human, cultured somatic cells they yielded hybrid cells capable of indefinite multiplication[8,9]. Applied to somatic cell protoplasts of two species of higher plants, they yielded hybrid protoplasts which regenerated, multiplied and developed into whole hybrid plants[10]. The list of cells and cell organelles fused has now expanded to include bacterial and fungal protoplasts and chloroplasts. It is reasonable to expect that PEG will induce the fusion of almost any two biological membranes and become an increasingly useful tool in their investigation[6,11].

We report here two features of PEG-induced fusion in cultured human fibroblasts. One is that fusion occurs preferentially between, or near to the tips of elongated processes. The other is that, contrary to previous interpretations[8,9], fusion occurs very near to, or even during, the time of PEG treatment.

The proportions of bi- and multinucleate cells at various times after pulse treatment with PEG of monolayers of early passage human skin fibroblasts were measured both by phase contrast microscopy[8,9] and by fixing and staining. The time sequence is as follows: about one fifth of the final proportion of bi- and multinucleate cells are clearly identifiable as such within about 30 min at 37 °C after treatment this proportion goes on increasing over a number of hours (6 to more than 24) to reach a peak of 30–60% (Fig. 1). The gradual decline after this peak is an artefact not relevant to the present paper. However, two of the factors contributing to it were ascertained: (1) unfused cells multiply, while very few cells with three or more nuclei do so; (2) a proportion of binucleate cells undergo the reverse of fusion, that is, cell division without prior nuclear division. The proportion of binucleate cells which go on to fuse their nuclei is too small to contribute appreciably to the decline in question.

The gradual rise in the proportion of bi- and multinucleate cells over a number of hours after treatment was interpreted[8,9] as indicating that fusion of treated cells could continue over many hours after removal of PEG. A first doubt on this interpretation arose from the following observation. In rather sparse fibroblast monolayers, where each cell sends out pseudopodia to touch other cells, configurations like those of Fig. 2 are commonly seen a few hours

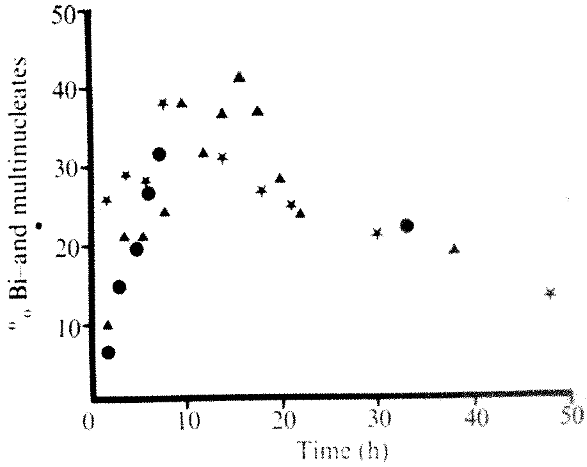

Fig. 1 Percentages of bi- and multinucleate cells at successive times after treatment of monolayers of human skin fibroblasts; PEG molecular weight 6,000, 50% (1w – 1v) in Dulbecco's medium for 1 min followed by washes in three progressively more diluted solutions of PEG and finally growth in Dulbecco's medium with 10% foetal calf serum. Different symbols represent different experiments. A variety of other effective treatments of monolayers with PEGs of different molecular weights, at different concentrations and with or without addition of dimethylsulphoxide[15] all show the same general trend: an initial increase with time of the proportions of bi- and multinucleate cells followed by a decline. For the meaning of this decline after the peak see text.

after treatment and before the peak proportion of bi- and multinucleate cells is reached. Points of contact between cells before treatment often involve a pair of thin elongated pseudopodia, one from each of two cells, or one pseudopodium from one cell in contact with the main body of another cell. Those pseudodopia which have fused following treatment later increase in diameter and contract in length. It takes time for two cells so connected to coalesce through an initially thin long connection. Two cells in the early stages of such coalescence would not be classified as a single binucleate cell. This mode of fusion is reminiscent of the fusion of myoblasts to form myocytes.

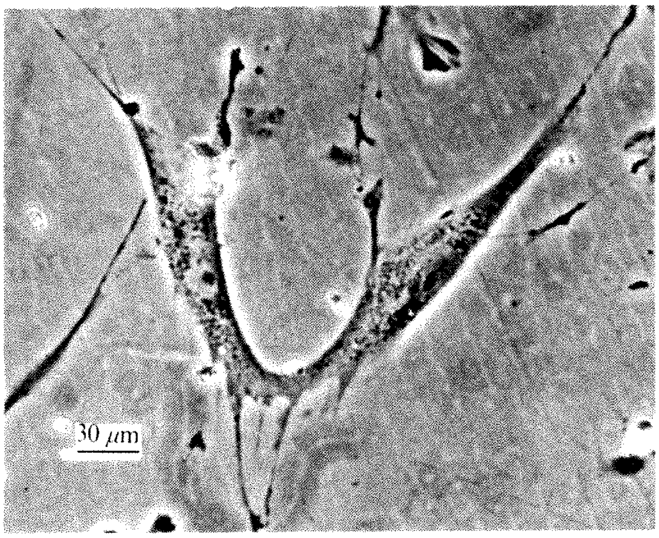

Fig. 2 Monolayer of human skin fibroblasts treated with PEG as in Fig. 1: a commonly seen configuration resulting from two cells which at the time of treatment fused at or near the tips of elongated filaments and gradually coalesced their cytoplasms. Phase contrast photography 3 h after treatment.

Time-lapse phase cinemicrophotography has provided an unequivocal answer. Applied to human fibroblasts in monolayer, treated for 1 min with 50% PEG (molecular weight 6,000), it has revealed three facts. First, some bi- and multinucleate cells are already classifiable as such soon after treatment. Second, apparently delayed 'fusions' are of the type just mentioned: two cells connected at the end of long thin pseudopodia coalesce their cytoplasms by slow contraction of these pseudopodia which fused near their tips at, or soon after, the time of treatment. The nuclei of such pairs of cells eventually come to lie next to each other in what has become a common cytoplasm, but this may take many hours. Third, time-lapse recording over more than 24 h of all the cells in individual fields has shown many cases of two cells initially far apart moving to touch each other. In no case, however, has fusion followed. If the increase with time of the proportion of bi- and multinucleate cells in monolayers were due to extensive fusion of such cells after removal of PEG we would have observed several such fusions.

Another kind of evidence comes from work on the time sequences of appearance of bi- and multinucleate cells in treated monolayers as compared with treated cell suspensions. The procedure developed by us and routinely used for treating cell suspensions[12] is to take up gently a well drained pellet of some 10^6 cells in 0.5 ml of PEG molecular weight 1,000 1w + 1.4v in Dulbecco's modified medium with 15% dimethylsulphoxide[13], dilute this suspension gently within 1 min with an equal volume of PEG molecular weight 1,000 1w + 3v without dimethylsulphoxide, and again with 4 ml of medium. This final suspension is then dispensed in flasks or dishes containing a volume of medium with serum at least 10 times greater. In contrast to treated monolayers (Fig. 1), the proportion of bi- and multinucleate cells classifiable as such from cells treated in suspension does not show the gradual rise in the first 24 h. The long time required by the cytoplasms of cells in monolayer to coalesce through thin long connections is not required by the rounded up cells of a suspension. It is important now to ascertain whether or not also in these cells fusion occurs prevalently between the tips of microvilli, or other microprocesses and, in general, between regions of high curvature.

Thus, as to the time of fusion, we conclude that whether two or more cells will ultimately coalesce and become recognisable as a single bi- or multinucleate cell is determined during or very near the time of treatment. In treated monolayers this final coalescence may take many hours, whereas in treated suspensions it occurs and can be recognised soon after treatment.

As to the mode of fusion, we conclude that in sparse monolayers it occurs mainly at or near the tips of filamentous processes of which every cell in low density cultures has many. This explains why the proportions of bi- and multinucleate cells found in treated sparse monolayers are as high as, or even higher than in confluent ones. In the latter, there is very extensive side-by-side contact between fibroblasts. Yet this does not lead to more fusion. It will be important to compare some of the physicochemical properties of the cell surface, such as charges, or distribution of intrinsic proteins, near the tips of processes with those on the main cell body (see for example refs 11 and 14). This preferential fusion near the tips of processes may well underlie the observation of a negative correlation between density of cells and proportion of mononucleate hybrids arising from PEG-treated mixed monolayers. This correlation was attributed to the lesser formation of large multinucleate cells in sparse monolayers. Instead it may well be the consequence, in part at least, of a greater fusion efficiency per cell in sparse monolayers due to the greater number of filamentous processes that fibroblasts show under this condition.

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Loss of duplicate gene expression after polyploidisation

INCREASES in cellular DNA content have been important in the evolution of eukaryotes[1–3]. One mechanism for increasing DNA content is polyploidisation, which has been observed in a wide variety of organisms[4–8], and which is believed to have preceded the evolution of the early vertebrates[9]. It has been suggested that, following gene duplication, many of the redundant copies would be silenced early in evolution by mutation[10,11]. Those duplicate genes remaining expressed are then available to diverge in structure and acquire new functions[9]. We present evidence of extensive gene silencing in the 50-Myr history of a family of tetraploid fishes. Furthermore, those species regarded by systematists as advanced (that is, morphologically divergent from the ancestral form) may have lost the expression of more duplicate genes than primitive species (that is, those with a morphology similar to the ancestral form).

Theoretically, the most useful group of organisms for the analysis of gene loss after polyploidisation would be one which has undergone polyploidisation relatively recently (before complete diploidisation has taken place), yet not so recently that the enzymes encoded by the duplicate genes are still indistinguishable. The Catostomidae meet these requirements and thus provide an excellent model system for investigating genome evolution after polyploidisation. Since their origin 50 Myr ago[12], sufficient time has elapsed for many changes at the levels of morphology and structural genes. At the chromosome level, however, all genera have conserved a tetraploid karyotype with $2n=100$, compared with $2n=50$ for most cypriniformes[12]. In addition, the DNA content of catostomid species examined is twice that of typical diploid fishes[12]. On the basis of morphology these catostomids have been placed in three subfamilies. Two primitive subfamilies (Cycleptinae and Ictiobinae) closely resemble fossil forms (*Amyzon*, Miocene) in morphology[13–15]. A single advanced subfamily (Catostominae) has diverged considerably in morphology. Starch gel electrophoresis of 20 enzymes was employed to reveal the extent of duplicate gene expression. The application of Nei and Chakraborty's formula[16] for the probability of electrophoretic identity of two proteins, with a time span of 50×10^6 yr, allows us to estimate conservatively that the isozymes encoded by duplicate genes will be separable by electrophoresis at least 95% of the time.

The distributions of duplicate genes expressed are presented in Tables 1 and 2. Considerable extinction of gene expression has occurred. For the entire family, the average loss of duplicate gene function is 53% (range 35% to 65%). The average percentage is similar to that observed in the few species of salmonid[17] and cyprinid[18] fish which have been studied. In many cases where two catostomid species have the same percentage of duplications expressed, different combinations of loci have undergone the diploidisation process. There is no obvious correlation with the metabolic role of an enzyme and the extent to which the duplicate loci encoding it have undergone diploidisation.

Several lines of evidence argue against the genes being silenced randomly throughout the entire 50-Myr period. It is commonly assumed that one of the duplicate loci is free to accumulate what would have been previously 'forbidden' mutations as long as the other locus remains functional. Can the drift to fixation of non-functional (null) alleles over 50 Myr account for the amount of diploidisation observed in the catostomids? It has been shown[11] that the probability, P, of a null allele drifting to fixation at a duplicate locus is given by

$$P=1-[4Nu+1][\exp(-ut)] \qquad (1)$$

If we assume a typical mutation rate $u=1\times10^{-6}$ per peptide per generation[19,20], the number of generations, $t=25\times10^{6}$, and a 'generous' estimate of an effective population size for a typical catostomid species, $N=10^{9}$, then it is calculated that $P>0.9999$. On the basis of this theory most duplicate loci should have been lost after a 50-Myr period, a prediction which is in disagreement with our observation that only 53% (on the average) of the duplicate loci have been lost. Although null alleles may have drifted to fixation shortly after polyploidisation, it is unlikely that they are currently doing so. The fact that polymorphisms for null alleles have not been observed in the catostomids suggests that fixation occurs rapidly. An additional consideration is that many of the duplicate genes have diverged in their tissue and developmental specificity and are differentially regulated[9,21]. We have observed many instances of such differential regulation. The duplicate Ldh-B loci in *Catostomus commersoni*, for example, are equally expressed in mature gonads, while only one locus is expressed in most other tissues. Because some of the duplicate loci have diverged in function and regulation, and because we have observed levels of duplicate gene expression much higher than predicted by random loss, it is likely that these duplicate genes are at present being either maintained or quenched by selection.

An evolutionary trend of diploidisation can best be seen by investigation of a large number of genera. One important inference generated by the present study is that species of the primitive subfamilies have retained more functionally duplicate genes than those of the advanced subfamily. An

Table 1 Number of duplicate genes expressed in the primitive catostomids

Species	N*	Ck-B	Sod	M-Mdh	Gpi-A	Gpi-B	G3Pdh	Ak-A	6Pgd	Ldh-B	S-Aat	Ald-C	Acp	Ck-A	Mdh-A	Mdh-B	Ldh-A	Ldh-C	Adh	Pgm	Xdh	% Gene duplications expressed
		Enzyme locus																				
Cycleptinae																						
Cycleptus elongatus	(15)	2	2	2	2	2	2	2	1	2	1	1	2	2	2	2	2	1	1	1	1	65
Ictiobinae																						
Ictiobus bubalus	(30)	2	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1	1	1	1	1	60
I. niger	(6)	2	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1	1	1	1	1	60
I. cyprinellus	(25)	2	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1	1	1	1	1	60
Carpiodes carpio	(25)	2	2	1	2	2	2	2	2	2	2	2	1	1	1	2	1	1	1	1	1	55
C. velifer	(20)	2	2	1	2	2	2	2	2	2	2	2	1	2	1	2	1	1	1	1	1	60
C. cyprinus	(25)	2	2	1	2	2	2	2	2	2	2	2	1	1	1	2	1	1	1	1	1	55

Average % of duplicate genes expressed = 59

The number of duplicate genes expressed for specific enzymes 50 Myr after tetraploidisation. The percentage of gene duplications still expressed is given for individual species of primitive catostomids. 2 indicates the presence of duplicate loci for an enzyme, 1 designates that only one locus is expressed. N* is the exact number of individuals examined for each locus for most species; however, for a few species, it represents an average among the loci studied. Late juvenile to adult fish were stored frozen, homogenised 1:2 (v/v) in Tris-HCl, pH 7, at 4 °C centrifuged 27,000g for 20 min, and the supernatant electrophoresed. Ak (adenylate kinase), Aat (aspartate amino transferase), Acp (acid phosphatases), Mdh (malate dehydrogenase—NAD), Pgm (phosphoglucomutase), and G3Pdh (glycerol-3-phosphate dehydrogenase-NAD) were resolved on histidine–NaOH buffer[25]; 6Pgd (6-phosphogluconate dehydrogenase–NADP), Ldh (lactate dehydrogenase–NAD), Ald (aldolase), and Sod (superoxide dismutase) on Tris-citrate[26]; Ck (creatine kinase), Gpi (glucose phosphate isomerase), and Sod on Tris–EDTA–borate[27]; Adh (alcohol dehydrogenase–NAD), and Xdh (xanthine dehydrogenase–NAD) on borate–NaOH[28]. Enzyme staining modified from Shaw and Prasad[29]. For most loci, various tissues were analysed. A diploid control, *Notropis stramineus*, showed no evidence of gene duplication.

Table 2 Number of duplicate genes expressed in the advanced catostomids

Species	N	Enzyme locus																				% Gene duplications expressed
		Ck-B	Sod	M-Mdh	Gpi-A	Gpi-B	G3pdh	Ak-A	6Pgd	Ldh-B	S-aat	Ald-C	Acp	Ck-A	Mdh-A	Mdh-B	Ldh-A	Ldh-C	Adh	Pgm	Xdh	
Catostominae																						
Erimyzon sucetta	(22)	2	2	2	1	2	1	2	2	2	2	1	1	1	1	2	1	1	1	1	1	45
E. oblongus	(25)	2	2	2	1	2	1	2	2	2	2	1	1	1	1	2	1	1	1	1	1	45
Minytrema melanops	(25)	2	2	2	2	2	1	1	1	1	1	2	1	1	1	2	1	1	1	1	1	35
Moxostoma erythrurum	(35)	2	2	2	2	1	1	2	1	1	2	1	1	1	2	1	1	1	1	1	1	35
M. duquesnei	(30)	2	2	2	2	1	1	2	1	2	1	1	1	1	2	1	1	1	1	1	1	35
M. macrolepidotum	(35)	2	2	2	2	1	1	2	2	1	1	1	1	1	2	1	1	1	1	1	1	35
M. cervinum	(35)	2	2	2	2	1	2	2	2	2	2	1	1	1	2	1	1	1	1	1	1	50
M. rhothoecum	(12)	2	2	2	2	1	2	1	2	1	1	1	1	1	2	1	1	1	1	1	1	35
Hypentelium nigricans	(30)	2	2	2	2	1	2	1	2	1	1	1	1	1	2	1	1	1	1	1	1	35
H. etowanum	(10)	2	2	2	2	1	2	1	2	1	1	1	1	1	2	1	1	1	1	1	1	35
Catostomus commersoni	(35)	2	2	2	2	2	2	2	2	2	1	1	1	1	2	1	1	1	1	1	1	50
C. catostomus	(17)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	45
C. occidentalis	(11)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	45
C. macrocheilus	(5)	2	2	2	2	2	1	1	2	2	1	1	1	1	2	1	1	1	1	1	1	40
C. columbianus	(14)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	45
C. luxatus	(2)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	45
C. plebeius	(25)	2	2	2	2	1	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	40
Chasmistes brevirostris	(1)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	1	45
Xyrauchen texanus	(11)	2	2	2	2	2	2	2	2	2	1	1	1	1	2	1	1	1	1	1	1	50

Average % of gene duplications expressed 42

The number of duplicate genes expressed for specific enzymes 50 Myr after tetraploidisation. The percentage of gene duplications still expressed is given for individual species of advanced catostomids. 2 indicates the presence of duplicate loci for an enzyme, 1 designates that only one locus is expressed. Abbreviations and methods are as explained in Table 1.

average of 59% of the loci show evidence of duplication in the primitives (12/20) compared to 42% in the advanced (8/20). Within the advanced subfamily, relatively primitive species such as *Moxostoma cervinum*[22] and *C. commersoni*[23] have more functionally duplicate genes than highly morphologically divergent species such as *M. rhothoecum* and *C. plebeius* (see Table 2). If the quenching of genes were completely random through time, or if tandem duplications occurred to any extent, the above relationship would not be evident. A phylogeny has been constructed, based on the comparison of the presence of one or two functioning gene loci, which yields a 'tree' similar in many respects with those based on morphology. From these considerations we conclude that species which are conservative at the morphological level are also conservative with respect to the retention of duplicate gene expression. In an analysis parallel to our study, Hinegardner[24] has found that the more generalised fish orders have higher cellular DNA contents than highly specialised orders. Our working hypothesis is that phenotypically advanced organisms lose more genetic information. After an initial DNA increase by polyploidy, the first stage of evolution will be a moderately rapid loss of the expression of redundant genes, as found in the catostomids. Then, over a longer period, this DNA which is not expressed is physically eliminated[24]. The catostomids are probably still in the first stage in view of their high DNA content.

In summary, during 50 Myr of post-polyploid evolution, the catostomids have returned to a functionally diploid state at over half the enzyme loci examined. This process is not entirely random. We suggest that some duplicate genes have been quenched more rapidly in the morphologically advanced species than in the morphologically conservative species.

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Common precursor pathways of Herpes DNA and of repair synthesis in ultraviolet irradiated cells

DEOXYRIBONUCLEOTIDE precursors of DNA synthesis may be supplied by two alternative pathways in mammalian cells[1]: (1) endogenous synthesis by reduction of the corresponding ribonucleotides at the diphosphate level, except thymidine nucleotides which are produced by deamination and methylation of cytidine deoxynucleotides at the monophosphate level; (2) exogenous salvage synthesis by stepwise phosphorylation of pre-existing deoxyribonucleosides. The first step leading to monophosphates is catalysed by purine[2] and pyrimidine[3,4] deoxyribonucleoside kinases. In cells infected with Herpes simplex type 1 (HSV) there is an induction of both thymidine kinase (TK)[5] and deoxycytidine kinase activity[6]. These two activities seem to be a property of the same virus-specified enzyme[7], whose role

Table 1 Effects of inhibitors of endogenous pathways on HSV production by ultraviolet-irradiated and non-irradiated stationary CV-1 cells

	Dose of ultraviolet (erg mm^{-2})			
	0	120	240	480
Treatment	Virus yield as % of the corresponding non-treated cultures			
None	100 (1.3×10^9†)	100 (3.1×10^8†)	100 (9.1×10^7†)	100 (2.3×10^7†)
Hydroxyurea, 10 mM	0.8–1.3‡	3.6	3.0	3.3–4.5‡
Cytosine arabinoside, 0.1 mM	0.2–0.4	0.65	1.4	2.7–3.5
Fluorodeoxyuridine, 2 mM	7–12	22	45	67–87
FUdR, 2 mM + thymidine 2 mM	64–71	—	—	187–207
Thymidine, 2 mM	55–70	—	—	123–144

Stationary CV-1 cultures (2×10^5 cells cm^{-2}) were kept before irradiation and infection with HSV in the presence of a minimum essential medium (MEM) without serum and tryptose phosphate broth, as previously described[10]. HSV was grown in the presence of MEM. The viral yield by control cultures was between 150 and 350 PFU per cell.

*The drugs were present during the 18 h of the viral cycle.

†PFU per Petri dish in the non-treated cultures.

‡Extreme values of three separate experiments.

remains unclear. It seems to be important for viral DNA synthesis in resting cells[5] in which the endogenous metabolism of DNA is low whereas the degradative phosphatase activity is high[9]. On the other hand viral TK activity increased faster during the exponential phase of viral DNA synthesis in cells irradiated with ultraviolet light before infection than in non-irradiated cells[10]. This suggested that viral TK may have a more important role in the synthesis of HSV DNA in ultraviolet-irradiated than in non-irradiated cells. I present here results showing that the exogenous pathways are preferentially used for both HSV replication and for DNA repair synthesis in ultraviolet-irradiated cells.

All experiments were performed in stationary (2×10^5 cells cm^{-2}) monkey kidney CV-1 cells. Culture, irradiation and infection of cells and the measurements of single cycle virus yield were carried out as previously described[10].

First, I measured the production of infectious virus by irradiated and control cultures in the presence of inhibitors of endogenous pathways such as fluorodeoxyuridine (FUdR) and cytosine arabinoside (ara-Cdr) which block the synthesis of deoxythymidilate[11] and of deoxycytidilate[12], respectively, and hydroxyurea (HU) which mainly inhibits the reduction of ribo- to deoxyribonucleotides[13,14]. Table 1 shows that viral production was more inhibited by these drugs in control than in irradiated cultures. The extent of inhibition of viral production by FUdR and ara-Cdr decreased as the dose of ultraviolet increased. Since both drugs must be phosphorylated in order to inhibit DNA synthesis[11,15,16], this effect could be due to a lower rate of phosphorylation of these inhibitors as a consequence of ultraviolet irradiation. I found that FUdR and deoxycytidine were phosphorylated to a lesser extent in extracts from ultraviolet-irradiated and infected cells than in extracts from controls (data not shown). HSV production was no longer impaired in the presence of both thymidine and FUdR (2 mM each) in non-irradiated cultures (Table 1). This might indicate that the exogenous supply of thymidine nucleotides is sufficient under these conditions to overcome the corresponding endogenous block induced by FUdR. However, one cannot exclude the possibility that viral and (or) cell TK have greater affinity for thymidine than for FUdR, leading to a lower rate of phosphorylation of the latter drug. In addition, Table 1 shows that in the presence of thymidine alone (2 mM) HSV production decreased in non-irradiated cultures and increased in irradiated cultures.

I measured in parallel the amount of incorporation of the four deoxyribonucleosides into DNA of uninfected cells in the presence of these inhibitors. This incorporation was more inhibited in control than in irradiated cultures (Table 2). The pool sizes of the free deoxyribonucleosides are unknown but probably differ in the two conditions since they depend on the rate of formation and of disappearance of deoxyribonucleosides which are both altered after irradiation as a result of the increase in nuclease activities leading to free deoxyribonucleosides[17] and by the depression of the rate of DNA synthesis[18]. As the deoxyribonucleoside concentrations used here (3×10^{-7} M) are sufficient to modify the natural pool size of the free deoxyribonucleosides occurring in normal cultures[19], it is not possible to obtain quantitative data from these last results. It can only be concluded that inhibitors of endogenous DNA synthesis are less effective (1) on the replication of HSV in ultraviolet-irradiated cells than in control cultures, (2) on the incorporation of the four deoxyribonucleosides into cellular DNA during post-ultraviolet DNA synthesis than during normal synthesis.

Table 2 Incorporation of deoxyribonucleosides into the DNA of normally growing and stationary ultraviolet-irradiated CV-1 cells in the presence of inhibitors of endogenous pathways

	Incorpotation as % of controls of:							
Treatment	Deoxyadenosine		Deoxycytidine		Deoxyguanosine		Thymidine	
	A*	B*	A	B	A	B	A	B
None	100 (9,975†)	100 (1,850†)	100 (3,360)	100 (1,060)	100 (10,810)	100 (2,620)	100 (19,320)	100 (3,990)
Fluorodeoxyuridine, 1 mM	48	86	32.6	49.5	46	60	43	117
Cytosine arabinoside, 0.1 mM	20.2	50	12.3	35	24.2	36	7	30
Hydroxyurea, 10 mM	15.1	55	33	360	12.7	51.5	4.9	30.6

CV-1 cells were grown in 3 cm diameter Petris dishes, then kept in the presence of each of the ^{3}H-deoxyribonucleosides at 2 μCi ml^{-1} + the four unlabelled deoxyribonucleosides to obtain a final concentration of 0.3 nmol ml^{-1} each, in MEM without serum (stationary) or with 5% dialysed newborn calf serum (growing). After 18 h, cells were rinsed three times with PBS, scraped into PBS, ultrasonicated (2 min at 4 °C), treated with DNAse-free pancreatic RNAse (20 μg ml^{-1}, 3 h at 37 °C) to digest the labelled RNA, then the 5% TCA-insoluble radioactivity was precipitated on to GF/C filters and counted as described[10]. The given values are the mean values of three Petri dishes.

*A, growing (3×10^4 cells cm^{-2}) cultures.

B, stationary ultraviolet-irradiated (240 erg mm^{-2}) cultures.

†Actual values for c.p.m. per Petri dish in the controls (mean values of three dishes).

The mechanism by which inhibitors of endogenous pathways decrease the incorporation of labelled deoxyribonucleosides by the exogenous pathways during normal DNA synthesis remains unknown. The cyclic increase of the TK activity at the beginning of the S phase[19] strongly suggests that the corresponding exogenous pathway has an important role in normal DNA synthesis, possibly as a salvage pathway[20]. An interrelated use of both endogenous and exogenous pathways could explain the mechanism mentioned above. In contrast, the excision-repair synthesis which occurs in these stationary ultraviolet irradiated CV-1 cells in the absence of normal DNA synthesis[10] would necessitate a low concentration of deoxyribonucleosides. This could be achieved by the exogenous pathways which are economical since (1) they permit the re-utilisation of the breakdown products arising from the irradiated DNA as previously shown[10], (2) they require only phosphorylating enzymes compared with the numerous enzymes involved in the endogenous pathways.

To test this last possibility I measured the level of the four deoxyribonucleoside kinases in extracts of stationary cultures made at different times after irradiation during the 48-h period[10], and found that the deoxyribonucleoside kinase activities decreased slightly by 6 h after irradiation, then increased, attaining a value at 24 h which was greater than that found prior to irradiation (Table 3).

Table 3 Phosphorylating activities of extracts of stationary CV-1 cells at different times after ultraviolet irradiation

^{3}H-precursor	Time (h) after irradiation with 240 erg mm^{-2}				
	0	6	12	24	48
Deoxycytidine	100 (1†)	62*	111*	140*	106*
Deoxyguanosine	100 (0.98)	77	122	128	110
Thymidine	100 (2.41)	85	119	141	126

Stationary cultures were kept in MFM without calf serum and tryptose phosphate broth between irradiation and extraction. Cell extracts were made and their enzyme content determined as described by Lin and Munyon[21]. For each cell extract at least three dilutions were tested over a range of protein concentrations where the reaction was linear (500–3,000 μg ml^{-1}). The final concentrations of the non-radioactive deoxyribonucleosides in the mixture reactions were, for deoxyadenosine and desoxyguanosine: 4×10^{-6} M; for deoxycytidine and thymidine: 2×10^{-5} M.

*The values are given as the percentage of the values obtained at time 0.

†nmol of deoxyribonucleoside phosphorylated per mg protein in 30 min at 38 °C.

The activity of deoxyadenosine kinase was too low to be detected in these conditions. The variations of the deoxyribonucleoside kinase activities observed after ultraviolet irradiation seem not to be due to the occurrence of any normal DNA synthesis, since similar results (unpublished observation) were obtained in extracts from cells kept in the presence of 2 mM of each of the four deoxyribonucleosides after irradiation, that is, in conditions where normal DNA synthesis must be inhibited by an excess of non-radioactive deoxyribonuclosides[22,23]. The increase of these kinase activities starting 6 h after irradiation could reflect either the ultraviolet-induced synthesis of new enzyme molecules, or the alterations of the activities of pre-existing enzymes, for example by small effector molecules like dTTP[19] leading to an end-product inhibition. Moreover, these results suggest a role of the deoxyribonucleoside kinases in DNA repair synthesis since their activities attain a maximum level at about the same time (24 h) at which DNA template function is fully restored, as shown by the recovery of interferon mRNA production[24] and of 45S ribosomal RNA transcription[25] in ultraviolet-irradiated stationary CV-1 cells.

In conclusion, it seems that deoxyribonucleoside precursors of DNA synthesis are supplied essentially by the exogenous pathways in post-ultraviolet DNA synthesis whereas in non-irradiated cells both exogenous and endogenous pathways are important in CV-1 cells. A similar pattern seems to occur for DNA synthesis of HSV grown in ultraviolet CV-1 cells as compared with virus grown in control cells. This conclusion is drawn from our earlier results[10] and from those obtained with inhibitors of endogenous pathways of cell and of viral[26,27] DNA synthesis. Such a pattern could explain that in the presence of thymidine at high concentrations, HSV production is slightly impaired in control cultures and increased in irradiated cultures (Table 1).

These results would indicate that the metabolic pathways are common to host cell and to HSV DNA synthesis. In this case, there might be a correlation between the residual ability to support HSV replication and the extent of repair synthesis in different ultraviolet-irradiated cell lines. I am currently investigating this possibility using normal skin cells and xeroderma pigmentosum cell lines which are defective in ultraviolet-induced repair synthesis.

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Maternal histidine metabolism and its effect on foetal development in the mouse

THERE have been reports[1,2] of possible deleterious effects on the mental development of children due to maternal histidinaemia, in which a deficiency of the enzyme histidase leads to the exposure of the foetus to abnormally high concentrations of histidine and its imidazole derivatives. This is analagous to the well established case of maternal phenylketonuria[3] which can lead to mental retardation in metabolically normal children. There is also a suggested link[4] between low maternal concentrations of two enzymes in galactose metabolism and infantile cataracts. We report

Table 1 Plasma amino acid values for different genotypes on various diets

Diet	*his/his*	Genotypes Histidine *his/+*	+/+	Genetic background
Normal	2.83 ± 0.18	0.17 ± 0.02	0.12 ± 0.007	50% Peru/50% C57
Normal + 2% His	6.08 ± 0.35	0.15 ± 0.06	0.13 ± 0.09	
Normal + 8% His	—	5.36 ± 0.48	0.45 ± 0.08	
'Low histidine'	0.44 ± 0.15	—	—	
Normal	2.97 ± 0.50	—	—	Peru
		Phenylalalanine		
Normal	—	—	0.098 ± 0.008	C57
Normal + 10% Phe	—	—	1.08 ± 0.09	
		Lysine		
Normal	—	—	0.26 ± 0.005	
Normal + 10% Lys	—	—	2.06 ± 0.62	
		Proline		
	pro/pro	*pro/+*	+/+	
Normal	1.92 ± 0.082	0.34 ± 0.007	0.36 ± 0.02	50% PRO:RE/50% C57
Normal + 2%	4.18 ± 0.87	—	—	

The normal diet was FFG/LAC (Fox Foods) ground into a powder and fed as damp mash *ad libitum* at 1700 h each day with or without additives (% by weight), histidine-HCl, phenylalanine, lysine, proline (Sigma). Because of the diurnal variation in feeding, the plasma values shown are not present during the whole 24 h. Blood was taken between 0900 and 1000 h. We have evidence that higher levels are present in the early morning and that by 1700 h the levels can drop to about 50%. The low histidine diet was a mixture of 75% histidine-free diet (HF-2, Cow and Gate) 25% normal diet plus 2% vitamin supplement (Nutritional Biochemicals). All determinations of amino acids were carried out by ion exchange column chromatography (ninhydrin) with α-guanidoproprionic acid as an 'external' standard added before deproteinisation by sulphosalyclic acid. All are expressed as μmol ml^{-1} with their standard errors.

here work on the nature of the maternal effects of histidinaemia using mutant mice which are the homologues of the human condition[5–7].

The activity of histidase is very low in the mouse mutant homozygotes (*his/his*<5% activity of +/+) resulting in up to 20 times the normal level of histidine and its derivatives in all tissues. Heterozygotes, with about 50% enzyme activity, have normal histidine levels on normal diets[5,6]. The histidinaemic mice have no overt abnormalities. On the other hand, some offspring of histidinaemic mothers, even when such offspring are metabolically normal, (*his/*+), display a balance defect resulting in circling behaviour which is associated with some or all of the following: deafness, damage during development *in utero*[5,6] to the canals, cochlea and otoliths of the inner ear.

We have now found that this maternal effect can also be produced by high histidine diet of pregnant mothers who, on normal diet, would give birth to normal offspring. We have also established that the middle third of pregnancy is the critical period. Conversely, it is possible to reduce the incidence of abnormal offspring of genetically histidinaemic mothers by reducing the histidine content of their diet during the second week of pregnancy. The exposure of the foetus to abnormal metabolic conditions of the mother, is, however, not the only determining factor for this condition. It seems that the genetic background can play an important role in influencing the susceptibility of the foetus to the metabolically generated teratogen. The murine maternal histidinaemia discussed here may well be of relevance to the aetiology of the homologous human abnormalities.

We were able to induce histidinaemia by purely dietary means. When heterozygous mice (*his*/×) are placed on an 8% histidine-supplemented diet the levels approach those of histidinaemic mice; homozygous wild-type mice (+/+) on this diet have only slightly higher levels than normal and were not used (Table 1). When we fed this diet to heterozygotes during the second week of pregnancy 14 balance defective offspring were produced out of 130 in 21 litters, compared with none in 209 animals on unsupplemented diet. No abnormal offspring were obtained from mothers fed the special diet during the first or third week. The critical period parallels that for *in utero* damage of the ear

Table 2 Effect of an 8% histidine-supplemented diet during the second week of pregnancy on the offspring of heterozygous (*his/*+) mothers

Diet	No. of offspring	Offspring genotypes	Effect on Balance	Effect on Hearing	Effect on inner ear Left Canals	Effect on inner ear Left Otoliths	Effect on inner ear Right Canals	Effect on inner ear Right Otoliths
(*a*) Offspring of mothers on 8% supplement	3	1 *his/his*; 2 *his/*+	Severe	Severe	Abnormal	Missing	Abnormal	Missing
	1	*his/his*	Mild	Mild	Normal	Missing	—	—
	1	*his/*+	Mild	Severe	Abnormal	Missing	Normal	Missing
	1	*his/his*	Normal	Mild	Normal	Missing	Abnormal	Missing
	2	2 *his/*+	Normal	Mild	Normal	Missing	Normal	Missing
	1	*his/*+	Mild	Normal	Abnormal	Altered	Abnormal	Altered
	2	2 *his/*+	Normal	Normal	Abnormal	Missing	Abnormal	Missing
	12	8 *his/his*; 4 *his/*+	Normal	Normal	Normal	Missing	Normal	Missing
	11	5 *his/his*; 6 *his/*+	Normal	Normal	Normal	Normal	Normal	Normal
(*b*) Offspring of untreated mothers	19	11 *his/his*; 8 *his/*+	Normal	Normal	Normal	Normal	Normal	Normal

The mice used were of 50% Peru/50% C57BL background. The treated and untreated animals were contemporaries so that variation in penetrance of the balance defect due to relaxation of selection (see text) was not relevant. The heterozygous (*his/*+) mothers were mated to homozygous (*his/his*) fathers so that the offspring listed above were 50% *his/his*, 50% *his/*+. Some of each genotype were affected. Balance was tested by spinning the animal by the tail and observing in a perspex cage. The animal was severely affected if it circled and mildly if it leant its head on one side[1,2]. Hearing was tested with a short, sharp high pitched noise when the animal was at rest. A severe effect was no reaction of the ears or body any time the animal was tested and mild was no reaction 50% of the time. It can be seen that an effect on balance is only observed when both canals and otoliths are affected.

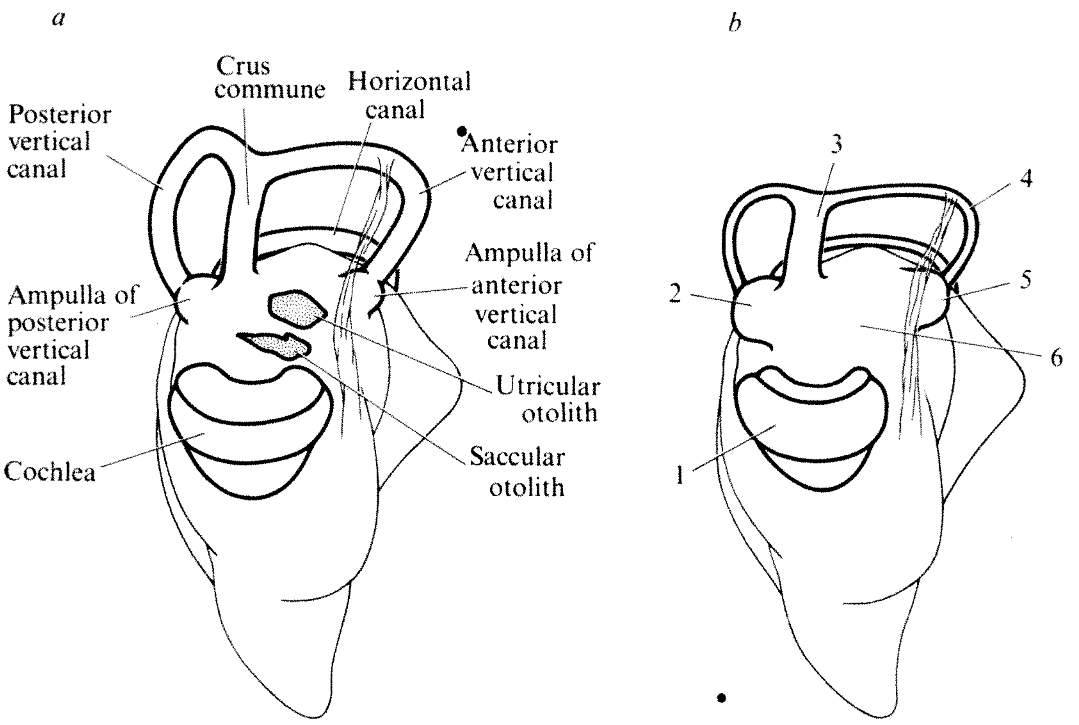


Fig. 1 Diagram of the abnormalities seen in the whole mounts of adult ears. *a*, Normal; *b*, affected. The ears were dissected into 75% alcohol and eventually cleared in benzyl alcohol. They were examined under the normal light and polarised light microscope. Canals are considered abnormal if they are shortened, thin or misshapen; otoliths are altered if they are enlarged or thin. About half the offspring from both treated and untreated mothers have utricular or saccular otoliths displaced but not damaged. This occurs in animals of the Peru or hybrid Peru/C57BL strains but not with pure C57BL. Any one ear may show one or more of the following abnormalities. 1, Enlarged or distorted cochlea; 2 and 5, enlarged ampullae; 3, shortened crus commune; 4, canals thin or misshapen; 6, otoliths absent, or thin or large crystals.

by X irradiation (9–16 d) (ref. 8). Details of the behaviour and effects on the vestibule of some of these offspring are shown in Table 2. The lesions in the balance defective animals are similar to those of balance defective offspring of genetically histidinaemic mothers[6] (Fig. 1). Some of the offspring without balance defects show subclinical levels of vestibular damage (Table 2*a*), as has been observed in some offspring histidinaemic mothers[6]. Offspring of untreated, metabolically normal females of the same stock show no clinical or subclinical lesions (Table 2*b*). Thus high maternal levels of histidine or its derivatives, however produced, are necessary for the production of balance defective offspring.

It is also possible to modify plasma histidine levels in histidinaemic (*his/his*) mothers during this critical second week of pregnancy. Histidine levels can be increased two to threefold further by a 2% histidine-supplemented diet and, of more importance, histidine levels can be reduced to about 10% (approaching *his*/+ or +/+ levels) by a low histidine diet (Table 1). The behaviour of offspring of mothers who received these diets is shown in Table 3. The 2% histidine-supplemented diet (which doubles the plasma histidine levels) did not significantly increase the proportion of balance defective offspring.

Table 3 Effect of a 2% histidine-suplemented diet, normal diet and low histidine diet, during the second week of pregnancy on the offspring of homozygous (*his/his*) mothers

Diet	No. of litters	No. of animals	Effect on balance Severe	Mild	Normal	% Affected	χ^2	P
(+)2% Histidine	23	79	12	10	57	27.8	1.15	ns
Normal	22	94	11	13	70	25.5	—	—
Low histidine	20	65	0	2	63	3.1	25.2	< 0.001

Genetic background of the animals, diet and scoring of the balance defect were as for Tables 1 and 2. The number of offspring with their balance severely affected, mildly affected and unaffected were tested by χ^2 test for both the 2% histidine-supplemented diet and the low histidine diet against the expected numbers on the normal diet.
ns, not significant

The histidine in untreated *his/his* mothers therefore, must lie above a 'saturation level' beyond which histidine levels are no longer relevant in determining the proportion of affected offspring. This conclusion is reinforced by the observations, previously described[6], that in any one litter there may be affected and unaffected offspring although all will have been exposed to the same maternal metabolites. In these circumstances other factors must be involved in the variation within and between litters. The low histidine diet, however, decreased the proportion of balance-defective offspring from 26% to 3%. In the lower range maternal histidine levels are therefore critical in determining the frequency and severity of balance defective offspring.

High levels of other amino acids (Table 1) during pregnancy do not have the same effect as histidine. Mice, homozygous for the gene *Pro-1*^{n} (refs 9–11) have <5% proline oxidase activity with consequent high levels of proline in their tissues. We have found that such prolinaemic mothers on a normal diet or fed a 2% proline-supplemented diet do not produce balance-defective offspring although their plasma proline is as high as plasma histidine in histidinaemics. Similarly, pregnant C57BL mice were fed on either 5% or 10% lysine or phenylalanine-supplemented diet in the, critical, second week of pregnancy. These diets increase plasma lysine or phenylalanine genes evolve independently in the model. Hence the probability $P_{N_0\to 0}(t)$ that all are extinct at time t is

$$P_{N_0\to 0}(t) = P^{N_0} = \left(\frac{t}{t+2N_0}\right)^{N_0} \qquad (12)$$

while the probability that only one survives, $P_{N\to 1}(t)$, is

$$P_{N_0\to 1}(t) = N_0(1-P_0)P^{N_0-1} = \frac{2N_0^2 t^{N_0-1}}{(t+2N_0)^{N_0}} \qquad (13)$$

The rate of change of the sum of these two probabilities gives the rate at which the number of surviving genes is reduced to one, that is the rate of gene fixation,

$$\frac{\mathrm{d}p_f(t)}{\mathrm{d}t}$$

Thus

$$\frac{\mathrm{d}p_f(t)}{\mathrm{d}t} = \frac{\mathrm{d}P_{N_0\to 0}}{\mathrm{d}t} + \frac{\mathrm{d}P_{N_0\to 1}}{\mathrm{d}t} = \frac{4N_0^2(N_0-1)t^{N_0-2}}{(t+2N_0)^{N_0+1}} \qquad (14)$$

With this rate, the cumulative probability of gene fixation by time t, $p_f(t)$, is

$$P_f(t) = \int_0^t \frac{\mathrm{d}p_f(t')}{\mathrm{d}t}\,\mathrm{d}t' = \frac{(2N_0^2+t)t^{N_0-1}}{(t+2N_0)^{N_0}} \xrightarrow[t\to\infty]{} 1 \qquad (15)$$

so that gene fixation always takes place and

$$\int_0^t \frac{\mathrm{d}p_f(t')}{\mathrm{d}t}\,\mathrm{d}t' = \frac{2N_0(N_0-1)t^{N_0}}{(t+2N_0)^{N_0}} \xrightarrow[t\to\infty]{} 2N_0(N_0-1) \qquad (16)$$

so that equation (11) gives the mean time to fixation in the birth and death model. Because one gene always becomes fixed, the probability of ultimate fixation of any particular gene is $1/N_0$.

Alternatively, Black and Gibson[29] measure the percentage of genes that become extinct. For example, from equation (9) the mean time it takes for 99% of the genes to become extinct is given by

$$0.99 = \frac{t}{2N_0 + t} \text{ or } t = 198N_0$$

If $N_0 = 100$, then one would expect that only one gene would remain after 19,800 crossovers. But since the mean value of $N(t)$ is N_0, given that one gene remained, one would expect to find it present in 100 copies. As Fig 3 shows, $P_0(t)$ rises rapidly, reaches 0.9 by $t = 18N_0$, and then slowly approaches its asymptotic value of 1.0. Thus this measure of the fixation time is very sensitive to the percentage criteria used when more than 90% extinction is specified. For example, while it takes 19,800 crossovers to attain 99% extinction of 100 genes, only 1,800 crossovers are required to reach 90% extinction Further, if one wishes to determine the dependence of the fixation time on N_0, a percentage fixation time is not suitable, for example, 99% elimination for $N_0 = 10^2$ and 10^6 leaves 1 and 10^4 remaining genes, respectively.

Estimates for the gene fixation time obtained from the birth–death model can be compared with Smith's[27] Monte-Carlo results. Smith lets $N(t)$ vary within set limits, chooses $p = 1/2$, and allows mispairing by a random number of positions as long as it is consistent with the prescribed latitude for $N(t)$. As a criterion for fixation he requires that the "clonality"

$$\left[\sum_{i=1}^{N_0}\left(\frac{n_i(t)}{N(t)}\right)^2\right]^{1/2} = 0.9$$

This criterion, which is roughly equivalent to requiring that 95% of the family be composed of copies of a single repeat, can be compared with requiring $n_i = 0.95\,N_0$ in our model Then the time at which $P_{0\,95N_0}$ is a maximum would logically correspond to Smith's fixation time. For the birth–death model this time, $t = (0.95N_0 - 1)N_0$ given by equation (6a), will vary quadratically with N_0 for large N_0. Smith found that fixation time increased approximately linearly with N_0. Further, say for $N_0 = 500$, Smith finds fixation times of the order of 10^4 crossovers, whereas the linear birth death model predicts $t \sim 2.4 \times 10^6$. Equation (11) which requires a more stringent fixation condition predicts $T_{bd} \sim 5 \times 10^5$. These discrepancies with Smith's simulations are due to the fact that random mispairings by more than one repeat were allowed in the simulations

Alternative models

In the model we have just considered, time has been measured in units of crossover events. If the number of crossovers within the multigene family per cell division were constant this approach would be appropriate One can, however, envision the situation in which the number of crossovers that occur per unit time is proportional to the number of homologous genes in the family Southern[35] has suggested that such a mechanism may account for the periodicities seen in mouse satellite DNA. Thus, suppose the rate of crossingover influencing gene i is proportional to n_i so that $\lambda_{n_i} = kpn_i$ and $\mu_{n_i} = k(1-p)n_i$, where k is the crossover rate per gene copy. For $p = 1/2$ this model is equivalent to the previous one with $N(t)$ replaced by k^{-1}. Thus it has the advantage of not requiring that $N(t)$ be approximated by its mean value, which for $p \neq 1/2$ would be time dependent. Hence, in this model, selective advantage (disadvantage) of a gene could be represented by using the known solutions to the linear birth–death problem[38] for $p > 1/2$ ($p < 1/2$).

A third model which leads to somewhat different results is to view the changes occurring at gene i as a gambler's ruin problem[38]. That is, if we assume that there exists some biological mechanism for maintaining the length of the multigene family constant, say at N_0, then gene i can contract until all copies are lost or it can expand until it attains length N_0. We may again assume that there are equal probabilities for gene i expanding and contracting by one copy, that is $p = 1/2$. If gene i attains length N_0, then all other genes are necessarily lost and gene i has been fixed. Since we have introduced no bias among the genes, the probability that gene i is fixed is $1/N_0$ and the probability that it is lost is $1 - 1/N_0$. When $p \neq 1/2$, as in a simulation of selective pressure, the probability of gene i being fixed is

$$\left(\frac{q}{p}-1\right)\Big/\left[\left(\frac{q}{p}\right)^{N_0}-1\right]$$

where $q = 1-p$ (ref. 38). As in the first model, one might suppose that the crossover probability per unit time for influencing genes i is n_i/N_0. Using results on conditioned random walks developed in another context[38], we can estimate the mean time to gene fixation, T_{gr}, measured in crossover events and determined by a gambler's ruin process, as

$$T_{gr} = \begin{cases} (N_0-1)N_0 & p = 1/2 \\ \dfrac{N_0}{|q-p|}\ln\dfrac{N_0 + \sqrt{N_0^2 + 4S(S-1)}}{2S} & p \neq 1/2 \end{cases} \qquad (17)$$

where $S = p/(p-q)$ for $p > 1/2$ and $S = q\,(q-p)$ for $p < 1/2$. Smith personal communication obtained the same conclusion for $p = 1/2$ using Kimura's results on the fixation of mutant genes, and W. A. Beyer and M. S. Waterman (personal communication) have shown the result to be asymptotically valid as N_0 gets large. The corresponding time for the first model with $p = 1/2$, obtained by setting the clonality to 0.9 is $t = (0.95\,N_0 - 1)N_0$, while the estimate obtained by setting $M_0(t) = N_0 - 1$ (see equation (11)) is precisely twice as long as that given by equation (17)

It is difficult to envision a biological process that would keep $N(t)$ constant If the fitness of an organism is maximal when $N = N_0$

crossover, with probability 1/2, one repeat will be eliminated, leaving no copies of it in the family. With the same probability some other repeat will be duplicated, leaving two copies of it. At a later stage in the process a repeat may have many copies, but there may still occur a streak of bad luck which leads to its elimination. Eventually, this will have occurred to all but one repeat. This repeat will then be fixed, and for $p = 1/2$, on the average, there will be N_0 copies of this repeat. As the process of unequal crossing over continues, the number of copies of this remaining repeat may also reach zero. In fact, the probability of this occurring will approach 1, but only as the number of crossovers approaches infinity. Since evolution only has had a finite time to proceed, this case is of no interest. Also, in reality, the loss of the genome would be highly selected against.

To assess the validity of replacing $N(t)$ by its mean value N_0 one can compare the results of Monte-Carlo simulations with our analytical results. Black and Gibson[29] performed such a simulation. They assumed that mispairing by one repeat occurs before each crossover, that $p = 1/2$, and they did not require that $N(t)$ remain constant. If one compares the Black and Gibson results for the percentage of the original gene types that remain in the family after a given number of crossovers with the predictions of the linear birth–death model one finds qualitative agreement (Fig. 2). The slight quantitative disagreement, especially at small values of N_0, may stem from our setting $N(t) = N_0$, or may be due to poor statistics in the Monte-Carlo simulations which start with few repeats.

In Fig. 3 we plot $P_{n_i}(t)$ for n_i between 0 and 4. For $n_i > 1$ the maximum of P_{n_i} occurs at

$$t = (n_i - 1)N_0 \tag{6a}$$

and has the value

$$(P_{n_i})_{\max} = \left(\frac{2}{n_i - 1}\right)^2 \left(\frac{n_i - 1}{n_i + 1}\right)^{n_i + 1} \tag{6b}$$

The probability of finding exactly n_i copies of the ith repeat decreases after $t = (n_i - 1)N_0$ partially due to the further duplication of this repeat. The probability $P_{n \geq n_i}(t)$ or finding n_i or more copies of the repeat is, for $n_i \geq 1$, given by

$$P_{n \geq n_i}(t) = \frac{2N_0}{t}\left[\left(\frac{t}{2N_0 + t}\right)^{n_i} - \left(\frac{t}{2N_0 + t}\right)^{N_0 + 1}\right] \tag{7}$$

which for $N_0 \gg n_i$ is approximately given by

$$P_{n \geq n_i}(t) = \frac{2N_0}{t}\left(\frac{t}{2N_0 + t}\right)^{n_i} = \left(1 + \frac{t}{2N_0}\right)P_{n_i}(t) \tag{8}$$

Since all repeats are ultimately lost, $P_{n \geq n_i}(t) \to 0$ as $t \to \infty$, but goes through a maximum, for $N_0 \gg n_i$ at $t = 2N_0(n_i - 1)$. This is shown in Fig. 4.

Fig. 4 The probability of having at least n_i copies of gene i at time t, measured in units of N_0 crossovers.

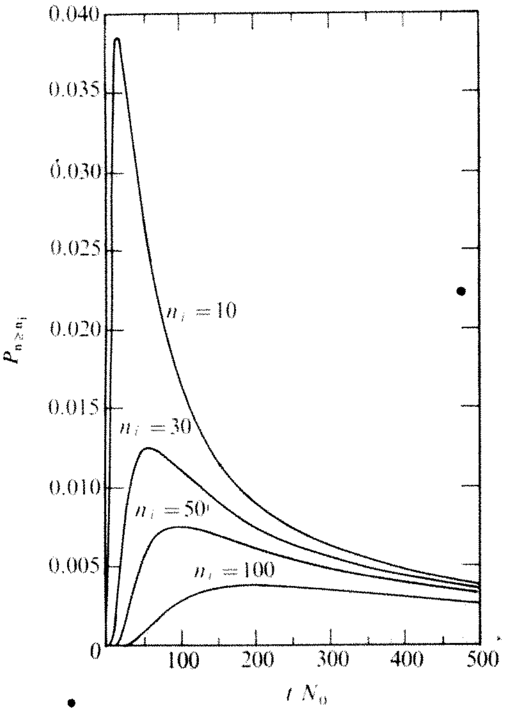


Table 1 Probability of generating multiple copies

n_i	t/N_0	P_{n_i}	$P_{n \geq n_i}$
1	0	1	1
>1	0	0	0
5	4	2.2×10^{-2}	6.6×10^{-2}
5	8	1.6×10^{-2}	8.2×10^{-2}
10	9	5.4×10^{-3}	3.0×10^{-2}
10	18	3.9×10^{-3}	3.9×10^{-2}
50	49	2.2×10^{-4}	5.5×10^{-3}
50	98	1.5×10^{-4}	7.4×10^{-3}
100	99	5.4×10^{-5}	2.7×10^{-3}
100	198	3.7×10^{-5}	3.7×10^{-3}
500	499	2.2×10^{-6}	5.4×10^{-4}
500	998	1.5×10^{-6}	7.4×10^{-4}

For some typical values of n_i, the number of repeats of the ith type, the probabilities $P_{n_i}(t)$ and $P_{n \geq n_i}(t)$ of finding exactly n_i copies and at least n_i copies, respectively, are given for $t = N_0(n_i - 1)$ and $t = 2N_0(n_i - 1)$. At these two times $P_{n_i}(t)$ and $P_{n \geq n_i}(t)$ attain their maximum values. In computing $P_{n \geq n_i}(t)$ we have assumed $N_0 \gg n_i$.

The probability of generating multiple copies of one particular gene by repeated unequal crossovers is surprisingly large, as illustrated in Table 1. Thus for $N_0 \gg 50$ in one out of every 135 organisms one gene would be replicated at least 50 times by unequal crossing over after $98N_0$ crossover events.

Gene fixation

Monte-Carlo simulations[27,29] have shown that after a large number of crossovers one gene tends to expand at the expense of the other genes in the multigene family. When a given gene comprises a large fraction of the family, its probability of subsequent loss is small, and one says that the gene has been 'fixed'. One can estimate the gene fixation time in two ways: let $M_e(t)$ be the mean number of extinct genes at time t. Because $P_0(t)$ is the fraction of genes that are extinct,

$$M_e(t) = N_0 P_0(t) = \frac{N_0 t}{2N_0 + t} \tag{9}$$

If

$$M_e(t) = N_0 - 1 \tag{10}$$

then the expected number of remaining genes is one. Combining equations (9) and (10) one finds that the expected fixation time, T_{bd}, determined by a birth–death process is

$$T_{bd} = 2N_0(N_0 - 1) \tag{11}$$

We have also computed the probability of one out of the original N_0 genes becoming fixed by time t, the probability of ultimate fixation, and show that the mean time to fixation in this birth and death model is precisely that given by equation (11). Our reasoning is as follows.

In the birth and death model we considered N_0 genes, each initially present in a single copy. The probability, $P_0(t)$, for any one of these genes to be extinct at time t was given by equation (5a). The

The coefficients $\lambda_{n_i}(t)$ and $\mu_{n_i}(t)$ can be identified as follows. As crossovers occur the number and order of genes on the chromosome may change. If there is an equal *a priori* probability of each repeat being influenced by crossover (that is duplicated or eliminated), then the probability of a particular gene, say gene i, being influenced by a crossover at time t is $n_i/N(t)$, that is, its fractional occurrence. Further we shall assume that the probability that a gene influenced by a crossover is duplicated equals p and the probability of its being eliminated is $(1-p)$. (In the absence of any selective force one would expect $p=1/2$.) Hence, measuring time by the crossover rate (so that one crossover is expected per unit time),

$$\lambda_{n_i}(t) = P\frac{n_i}{N(t)} \tag{3a}$$

$$\mu_{n_i}(t) = (1-p)\frac{n_i}{N(t)} \tag{3b}$$

Notice that

$$N(t) = \sum_{i=1}^{I} n_i(t)$$

is a random variable. With the birth and death parameters given in equation (3), the probability of having n_i copies of gene i will depend on the number of copies of all the other genes. To proceed we shall replace $N(t)$ by its mean value, which for $p=1/2$ is N_0. Thus we are approximating the birth and death rates λ and μ by their mean values. For other values of p, the mean value of $N(t)$ can be determined by considering a non-symmetrical random walk beginning at N_0 with an absorbing boundary at zero. In either event there is a finite probability for the loss of all the genes in the family; for $p \leq 1/2$ the ultimate loss is certain, while for $p > 1/2$ the extinction probability is

$$\left(\frac{1-p}{p}\right)^{N_0}$$

(ref. 38). When $p<1/2$ the expected lifetime of the family, measured in crossover events, is $N_0(1-2p)^{-1}$ (ref. 38). For $p=1/2$ the expected lifetime is infinite, while for $p>1/2$ the genes in the family may never be eliminated[38].

Under the assumptions $p=1/2$ and $N(t)=N_0$,

$$\lambda_{n_i} = \frac{n_i}{2N_0} = \mu_{n_i}, \qquad n_i = 0,1,\ldots N_0 \tag{4}$$

and equations (1) with initial conditions (2) reduce to the classical linear birth–death problem. The explicit solution, which may be found in Feller[38], is

$$P_0(t) = \frac{t}{t+2N_0} \tag{5a}$$

$$P_{n_i}(t) = \frac{(t/2N_0)^{n_i-1}}{(1+t/2N_0)^{n_i+1}}, \qquad n_i \geq 1 \tag{5b}$$

Observe that as $t \to \infty$, $P_0 \to 1$ and $P_{n_i}(t) \to 0$, and hence all genes in the family will ultimately be lost. It is easy to verify that

$$\sum_{n_i=0}^{\infty} P_{n_i}(t) = 1$$

that the mean value of n_i,

$$M_1(t) = \sum_{n_i=0}^{\infty} n_i P_{n_i}(t) = M_1(0) = 1$$

for all finite t, and that the variance $M_2(t) = t/N_0 + 1$. Hence for all finite times each of the original N_0 genes has a mean occurrence of one and a variance that increases linearly with time.

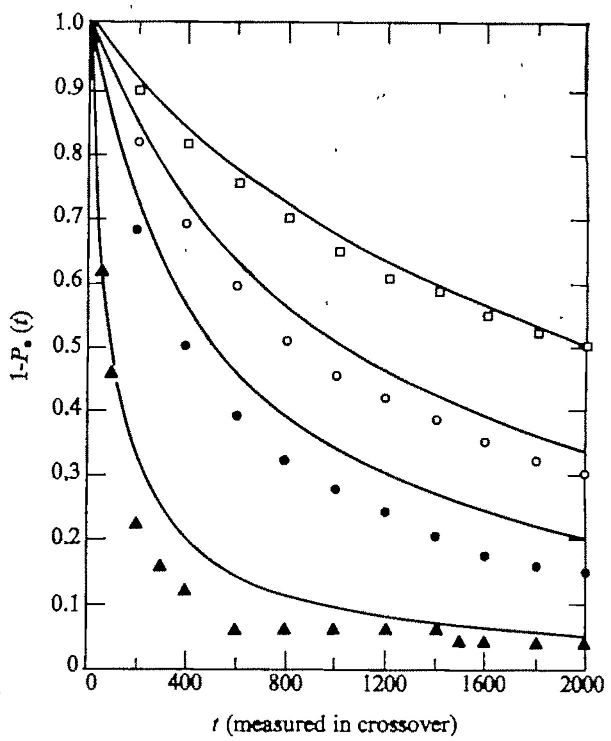
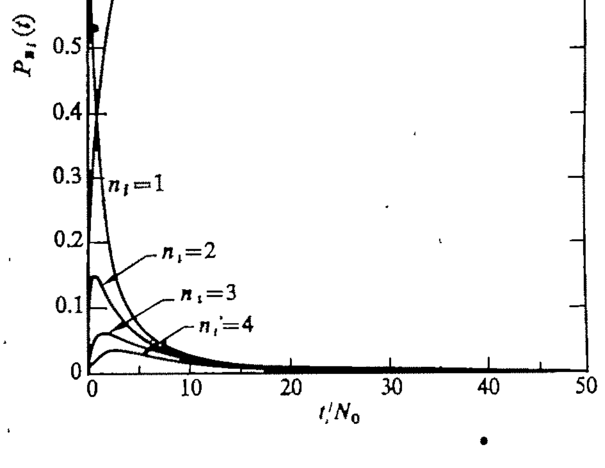


Fig. 2 Comparison of the predictions of the linear birth–death model (solid lines) with the Monte-Carlo simulations of Black and Gibson[29] (data points) The effect of the number of unequal crossovers and the size of the initial gene set on the rate of gene fixation. ▲, $N_0=50$; ●, $N_0=250$, ○, $N_0=500$; □, $N_0=1{,}000$

Even though the probability of any repeat being lost approaches one as $t \to \infty$, not all repeats will be lost in finite time, and in fact, one repeat will become fixed. One can understand this behaviour as follows: at $t=0$ there is one copy of each repeat At the first

Fig. 3 The probability of having n_i copies of gene i at time t, measured in units of N_0 crossovers for n_i between 0 and 4.



proven useful in the elucidation of a possible mechanism of gene magnification and reduction[2,13]. Restriction enzyme analysis and nucleic acid hybridisation have shown that about 50% of the DNA in the human Y chromosome is a simple tandemly repeated DNA (ref. 14).

It has been suggested[1,15] that sets of duplicated genes form multigene families, that is, are a group of nucleotide sequences or genes that exhibit the properties of multiplicity, close linkage, sequence homology and related or overlapping phenotypic functions. In particular, it has been argued that the genes that code for the variable regions of antibody molecules, V_λ, V_κ, and V_H, form three unlinked multigene families[1,15–18].

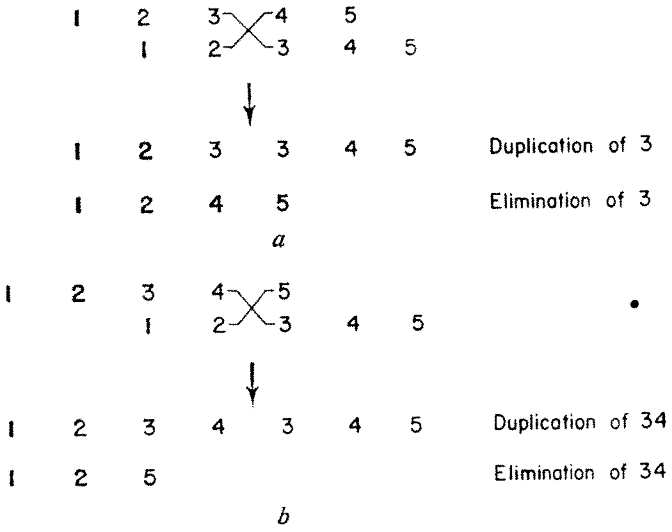


Fig. 1 Unequal crossing over between tandem sets of homologous genes and the products of the events. *a*, Mispairing by one gene; *b*, mispairing by two genes.

Genetic mechanisms need to be found which can not only generate repetitive gene sequences, but also explain the evolution of multigene families. Although the simplest hypothesis is to assume that the repeats in a multigene family evolve independently, there is evidence to the contrary. The genes within a multigene family can be maintained virtually identical within a species, and yet show divergence between species. This is the case with the spacer portions of the rRNA genes and the species-specific residues found in the conserved portion of the antibody V region that distinguish most immunoglobulins of one species from those of a second[19,20]. This unusual evolutionary property of multigene families termed coincidental evolution[1], species-specific evolution[19], horizontal evolution[21] and co-evolution[22], could be explained by parallel evolution, a gene correction process such as Callan's master slave hypothesis[23] or Gally and Edelman's democratic gene conversion theory[22], or, alternatively, a process of repeated duplication of some genes in a family and a loss of others[1,19]. Here we shall not discuss which of these proposals is most likely (for such discussion see refs 1 and 22), but we wish to point out that Tartof[2,13,24] has evidence supporting the hypothesis that unequal mitotic sister chromatid exchange is responsible for the magnification and reduction of rDNA in *D. melanogaster*, and that some proponents of the germ line theory of antibody diversity have suggested unequal crossing over as a mechanism of formation for species-specific V region subgroups[19,25–29]. Unequal crossing over has also been invoked to explain the origin of abnormal haemoglobulins such as haemoglobulin Lepore[30] and haemoglobulin Gun Hill[31,32], the variations in the length of the α chain of human haptoglobin[33], and the existence of the human γ-globulin hybrid γG3–γG1[34]. Southern[35] suggested that unequal crossing over might also explain the long-range periodicities found in mouse satellite DNA.

Smith[27,28], Black and Gibson[29], and Tartof[24] have used Monte-Carlo computer simulations to study the evolution of multigene systems through unequal crossing over. They have found that after large numbers of crossovers, one gene in the multigene family tends to become fixed while all other genes are lost asymptotically.

Monte-Carlo simulations can only deal with limited numbers of genes. Thus there is no way to be certain that results obtained on multigene families with say 10^3 repeats hold for simple-sequence families which may contain as many as 10^7 repeats of a single nucleotide sequence. If unequal crossing over is to be a credible theory for the evolution of multigene families, large families need to be studied. Here we develop a simple mathematical model of sister chromatid crossing over which yields predictions consistent with the Monte-Carlo simulations and which allows the computation of fixation times for a family of any size.

Crossing over can occur either at meisosis or mitosis[36]. Sister-chromatid exchanges have been observed to occur at frequencies of the order of 0.2 per chromosome per mitosis[37]. During unequal, intrachromosomal crossing over, sister chromatids carrying closely linked homologous genes mispair, crossover and produce two new sister chromatids, one containing a greater and the other a lesser number of gene repeats (Fig. 1). A mispairing by one position before the crossover yields a duplication of a single gene in one chromatid and the elimination of the same gene in the other chromatid, while a mispairing by k positions leads to the duplication and elimination of k genes. Thus, in so far as the state of the genome is determined by the number of copies of each gene and does not depend on gene order, any state attained by unequal crossing over can be realised by a sequence of unequal crossover events where the mispairing is by one repeat.

Mathematical model

Assume that at time $t = 0$ there are N_0 adjacent homologous DNA sequences which represent a multigene family. These sequences need not be identical, only similar enough to allow mispairing. We also assume that of the N_0 sequences I are distinct sequences ($I \leq N_0$), called "genes". These distinct sequences need not represent a classical gene but rather may represent a repeat unit containing multiple genes as in the case of *Xenopus* rDNA. We shall follow one of these I genes, say gene i, through a sequence of crossover events and determine the probability, $P_{n_i}(t)$, of there being n_i copies of this gene at some later time t, We assume that at $t = 0$ each gene, including gene i, is present in a single copy so $I = N_0$. For $t \neq 0$, the total number of homologous sequences, $N(t)$, is a random variable which need not be equal to N_0. Restricting our attention to mispairings by one repeat at any crossover events and determine the probability, $P_{n_i}(t)$, of there being n_i copies of this gene at some later time t. We assume that at one. If the probabilities of these events per unit time are $[1 - \lambda_{n}(t) - \mu_{n_i}(t)]$, $\lambda_{n_i}(t)$ respectively, a birth-death model[38] yields the following equations:

$$\frac{dP_{n_i}(t)}{dt} = -[\lambda_{n_i}(t) + \mu_{n_i}(t)]P_{n_i}(t) + \lambda_{n_i-1}(t)P_{n_i-1}(t) + $$

$$+ \mu_{n_i+1}(t)P_{n_i+1}(t),$$

$$n_i \geq 1, \qquad i = 1,2,\ldots N_0 \qquad (1a)$$

$$\frac{dP_0(t)}{dt} = -\lambda_0(t)P_0(t) + \mu_1(t)P_1(t),$$

$$n_i = 0, \quad i = 1,2,\ldots N_0 \qquad (1b)$$

with the initial conditions

$$P_{n_i}(0) = \begin{cases} 1 \text{ for } n_i = 1 \\ 0 \text{ for } n_i \neq 1 \end{cases}, i = 1,2,\ldots N_0 \qquad (2)$$

cells expressing a single Ig may produce one new mutant in the combining site every generation—say 12 h. A population of 10^8 such cells (an average *Nature* reader has 10^{12} lymphoid cells) could thus generate 200 mutant genes per day. This number compares with current estimates of the total number of germ line antibody genes.

In summary, stable, spontaneous, somatic mutations have been detected in antibody genes in the absence of antigen or other avoidable selective pressure. The observed mutations have been apparently non-random. The mutation rate may be high enough to generate the diversity required from the germ line genes. Although our results do not directly implicate somatic mutation as a mechanism for antibody diversification *in vivo*, it seems likely that somatic mutation, coupled with antigen selection of new antibody combining sites, has an important biological role.

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articles

Mathematical models for the evolution of multigene families by unequal crossing over

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Mathematical models of homologous but unequal crossing-over between sister chromatids are presented. For mispairing by one repeat, the evolution of a multigene family by unequal crossing over can be represented by a linear birth–death process. The fixation rate of one repeat in a multigene family is estimated. For mispairing by more than one repeat, some approximate results are obtained.

EVIDENCE obtained during the past decade, reviewed in refs 1 and 2, has suggested that the eukaryotic genome contains multiple copies of certain nucleotide sequences, including genes. For example, satellite DNA isolated from *Drosophila virilis* contains about 10^7 copies of a heptanucleotide repeat known as satellite sequence I. Each cell also contains more than 10^6 copies of satellite sequences II and III, heptanucleotide repeats which could be derived from sequence I by single base substitutions. The gene coding for 28S and 18S ribosomal RNA (rRNA) in various eukaryotes has been found to be randomly repeated[2]. In the amphibian *Xenopus laevis* about 450 repeating units are clustered at the nucleolar organiser. Each unit has three major regions: 18S, 28S and spacer[3–5]. The spacer regions, which are not transcribed[6], are composed of a series of small tandem homology units. The number of these homology units differs from spacer to spacer and hence the spacers are heterogeneous in length[8,9]. Except for the spacer regions the 450 ribosomal units of *X. laevis* are identical to one another within the accuracy of DNA–DNA hybridisation techniques[4,7,10]. Comparison of the ribosomal repeats of *X. laevis* with its sister species *X. mulleri* shows that the 18S and 28S genes of the two species are indistinguishable, but the spacer regions differ widely in nucleotide sequence[4]. In *Drosophila melanogaster* tandemly repeated genes coding for rRNA (rDNA) are also clustered at the nucleolar organiser[11,12]. There is one nucleolar organiser on each X and Y chromosome. A mutant known as *bobbed*[12] has been isolated that is partially deficient in rDNA genes, and hence, has mutations at the mouse γ1 structural gene locus. They originate from four different mutation events (Table 1). Two of these seem to be point mutations—IF1 (a nonsense) and IF4 (a mis-sense). IF3 is a frameshift mutation resulting from a two-base deletion. The large deletion of IF2 may be due to a translocation or a mitotic recombination. All these processes have been well characterised in bacteria and lower eukaryotes. The studies described above show that they can take place in mammalian somatic cells. A statistical analysis of their relative frequencies is obviously premature. More examples may give some indication as to whether the greater complexity of the mammalian genome is associated with an alteration in the relative frequencies of the mutation events. In general, studies on mutations of somatic cells have involved mutagens. An important question is whether these agents merely increase mutation rates, or cause mutations that rarely (or never) occur spontaneously, or both. The studies above, although very restricted, provide a background on the more neglected area of spontaneous mutation. The procedures described above can equally be used to characterise the structural alterations induced by mutagens, particularly carcinogens.

Table 1 Structural mutants of MOPC 21 (P3) mouse myeloma. The molecular weights are calculated from their sequences allowing 2000 daltons for carbohydrate

	Wild type	Mutants			
	MOPC 21	IF1	IF2	IF3	IF4
Molecular weight	50,800	41,300	40,900	40,700	50,800
H-chain defect		Deletion of C_H3 (residues 358–440)	Deletion of C_H1 (residues 121–214)	Altered sequence of residues 341–354 followed by deletion of C_H3	Asparagine to aspartic acid (residue 415)
Sialic acid	−	+	−	−	−
Binding to F_c-receptor[48]	+	−	+	−	N.D.
H-chain mRNA size	17S	17S	16S	17S	17S
Proposed genetic mechanism		Point mutation ('nonsense')	Intracistronic deletion	Frameshift (−2) Premature 'ochre' termination	A to G transition ('mis-sense')

N.D. not determined.

Somatic mutation and antibody diversity

There were about 300 generations between the isolation of the P3 clone and the detection of mutants. Since five mutants were isolated from 7,000 subclones, the electrophoretic mutation rate of H-chain genes can be calculated, using the Catcheside approximation[39] as 3.3×10^{-6} per cell per generation. This mutation rate is higher than those reported for spontaneous auxotrophic mutants of Chinese hamster ovary cells in culture[6] or for spontaneous variations in other mammalian cell systems[12,17], which are less than 10^{-8} per cell per generation.

From the H-chain sequence and the genetic code, we have calculated that 23% of all theoretically possible point mutations (two out of four in our case) will lead to a charge change[29]. The corrected mutation rate (to include electrophoretically silent mutations) approaches 10^{-5} per cell per generation. This calculated rate must be a conservative estimate for several reasons. Certain mutations might lead to distortions in the protein structure causing problems of solubility and resulting in either a rapid catabolism[40] of the protein, or in cell death. In others, the mutant polypeptide chain might be too small to be detected by our methods. The low levels of mutant polypeptide chain might be too small to be detected by our methods. The low levels of mutant proteins found in the sera of tumour-bearing mice may be a result of any of these processes of selection.

The striking similarity between IF2 Ig and the human heavy-chain disease proteins[41] has been stressed before[22] and may be related to the mechanism of integration of V and C genes. The point mutation in IF4 also occurs in an interesting residue. Variants of human kappa and lambda chains have been located in a homologous region. Residue 415 of MOPC 21 H chain, the O_z marker of lambda chains[26], and the Inv marker of kappa chains[26] are, respectively, four, four and three residues before the second pseudosubunit half cystine. The mutations in IF1, IF2 and IF3 are all outside the pseudosubunit disulphide loops although more residues (247 residues) are within than outside (193 residues) the pseudosubunit disulphide loops (Fig. 4). This apparent bias in favour of mutations outside the loops could be the result of strong selective pressure at the protein level against mutations (or deletions) within the disulphide loops. In addition, normal mutation rates may be dependent on the DNA sequence[42,43] and there might be mutational 'hot spots'. This is particularly applicable to the deletion of IF2 which starts at the V–C junction and ends at the residue repeatedly involved in deletions[41]. Selection might also occur at the mRNA level, against mutants with less than optimum base-pairing arrangements. Such mutants could lead to mRNA instability resulting in the loss rather than the alteration of chains. The possibility that experimental immunoglobulin mutations may occur non-randomly along the chain has implications for the antibody diversity problem.

The functions of the immune system include the recognition of, and response to, an apparently limitless array of antigens. Variability in each Ig class or subclass is confined to the V regions, especially in the 50–70 residues that form the antigen-binding sites[44] in the V_H–V_L domain: the hypervariable residues. Recent results on quantification of the number of germ line genes by molecular hybridisation methods indicate that somatic mutation of antibody genes has a role in the diversification of Ig binding site sequences (reviewed in ref. 45). In the mutations reported here the V regions have not been involved. This may be due to the small number of mutants studied but it may be also that our cell line, which is already committed to Ig production, behaves differently from antigen-sensitive virgin cells. Cunningham and coworkers[46] have suggested that most combining site diversity occurs in a clone of cells after, and as a result of, antigen contact. If this is so, higher mutability should be expected on antigenic stimulation. However, the stability of a clone of antibody-producing cells *in vivo*[47] does not support such a view. Even so it is possible that uncommitted cells have a higher mutation frequency in their hypervariable region than more mature cells. We have suggested that some of the germ line genes include DNA sequences signalling higher mutation rates[50]. This advantage may decline as mutants develop in the direction of more stable configurations.

The conservative mutation rate calculated here for the whole IgG is about 10^{-5} per cell per generation. Since hypervariable regions represent only 10% of the total molecule a mutation rate of 10^{-6} per cell per generation will affect the combining site. This means that even in committed cells a clone containing 10^6

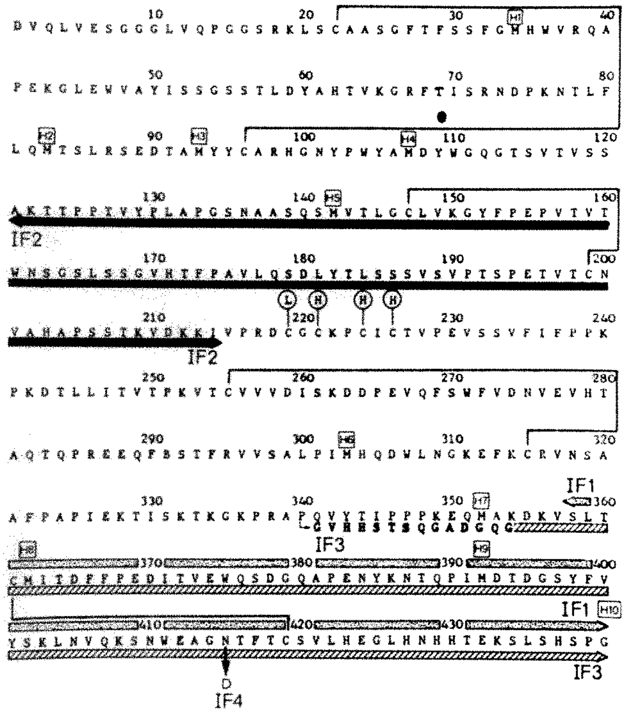


Fig. 4 The sequence of MOPC 21 H chain and of its mutants. The horizontal arrows indicate the extent of the deletion in each mutant. For IF3, an altered sequence continues for 14 residues beyond Pro-340 (see Fig. 5), and for IF4, Asn-415 is replaced by an Asp. H1, H2, and so on indicate the C-terminal residue of the respective CNBr fragments of MOPC 21 H chain.

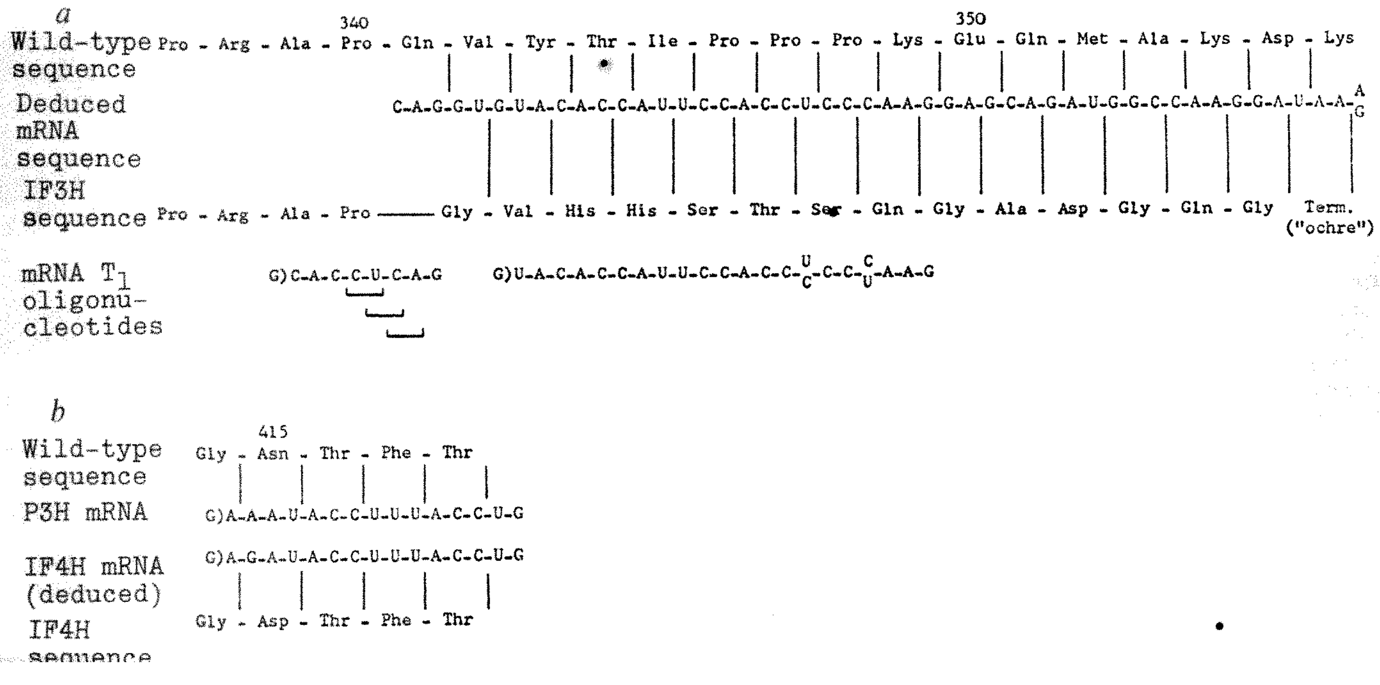
29, 32) was the electrophoretic mobility (*p*H 6.5) of the ^{14}C-carboxymethylcysteinyl peptide H10T3 (ref. 34), residues 409–433 (Fig. 3) Thermolysin digestion of this peptide showed that while in wild-type Asx-415 was present in the neutral peptide Ala-Gly-Asn-Thr, in IF4 H chain it was in the acidic peptide Ala-Gly-Asp-Thr. The difference in structure between wild-type and mutant IF4 is therefore an asparagine–aspartate interchange at residue 415. (Pulse-labelled (15-, 30- and 60-min pulses) intracellular wild-type and IF4 immunoglobulins showed the same charge difference as the two proteins isolated from sera, indicating that the difference is not a result of deamidation. Further evidence on this point was obtained by fingerprint analysis of the H-chain mRNA from mutant cells. An oligonucleotide obtained by digestion with T$_1$-ribonuclease (h6, Fig. 5), which was proposed to code for residues 414–419, had been previously isolated from ^{32}P-H-chain mRNA (ref. 31). The Asn → Asp interchange at 415 implies an A → G transition and therefore a split in oligonucleotide h6 at the new G residue. Fingerprints of IF4 ^{32}P H-chain mRNA showed that h6 was not present in the mutant. Unfortunately the predicted alternative oligonucleotide could not be positively identified. It is smaller than the wild type form and likely to be obscured by other oligonucleotides. The evidence indicates that the Asn–Asp interchange is the result of a point mutation.

Significance of the mutations

The mutations described above are not due to post-translational modifications. This was established by translation *in vitro* and structural studies of isolated mRNA. The possibilities that the mutant proteins are all due to post-transcriptional modifications of RNA or derived from genes silent in wild-type cells but expressed in the mutants, are not formally excluded but are most unlikely for several reasons. All the mutations are consistent with simple genetic events (Table 1) and no other primary sequence differences were detected. Each mutant represents a unique sequence, not observed in other myeloma proteins. Therefore, at least four, but probably more, such silent genes would have to be postulated. In addition, immunoglobulin V and C genes require an integration process for the expression of the mRNA[26,35]. This integration occurs during differentiation of stem cells into plasma cells[36,37]. The expression of a silent C gene would require a retranslocation of the V gene, since the V region of the mutants is the same as that of the wild type. Translocation of V genes onto new C genes in somatic cells *in vivo* gives rise to the expression of other known H-chain classes[26]. This type of translocation has not been observed with plasma cells in culture. Attempts to detect them in established cell lines have been unsuccessful[32]. In other cases, however, it has been suggested that a modified C gene, with characteristics of another subclass, was detected in chemically-induced mutant cells as a result of deletion or unequal crossing over[38].

We conclude that IF1, IF2, IF3 and IF4 represent spontaneous

Fig. 5 The sequence of two stretches of wild-type heavy chain and of mutants. *a*, Sequence of the wild-type[22] and IF3 H-chain can be read from a single mRNA sequence assuming a deletion of two bases, giving rise to a frameshift. Alternative positions of the deletion are indicated. *b*, Alteration of the point mutant IF4. T$_1$ oligonucleotide sequences taken from ref. 31.


a
340
350
Wild-type sequence
Pro - Arg - Ala - Pro - Gln - Val - Tyr - Thr - Ile - Pro - Pro - Pro - Lys - Glu - Gln - Met - Ala - Lys - Asp - Lys
Deduced mRNA sequence
C-A-G-G-U-G-U-A-C-A-C-C-A-U-U-C-C-A-C-C-U-C-C-C-A-A-G-G-A-G-C-A-G-A-U-G-G-C-C-A-A-G-G-A-U-A-A-G
IF3H sequence
Pro - Arg - Ala - Pro —— Gly - Val - His - His - Ser - Thr - Ser - Gln - Gly - Ala - Asp - Gly - Gln - Gly
Term. ("ochre")
mRNA T1 oligonucleotides
G)C-A-C-C-U-C-A-G
G)U-A-C-A-C-C-A-U-U-C-C-A-C-C-U/C-C-C-C/U-A-A-G
b
415
Wild-type sequence
Gly - Asn - Thr - Phe - Thr
P3H mRNA
G)A-A-A-U-A-C-C-U-U-U-A-C-C-U-G
IF4H mRNA (deduced)
G)A-G-A-U-A-C-C-U-U-U-A-C-C-U-G
IF4H sequence
Gly - Asp - Thr - Phe - Thr

Thus, the 94 residues from 121 to 214 of the wild-type chain were missing from the sequence of this mutant and a new bond corresponding to Ser$_{120}$-Val$_{215}$ was found. The amino acid analysis of all CNBr fragments of IF2 H chain, the peptide maps and the amino acid compositions of the tryptic peptides[29], were consistent with the proposed internal deletion of 94 amino acid residues (Fig. 4).

The mRNA prepared from this mutant can be translated in cell-free systems to give a mutant heavy chain. Unlike IF1, the mRNA of IF2 does not comigrate in sucrose gradient sedimentation with the wild-type H-chain mRNA, but is significantly smaller. The decrease in size, from 17S to 16S, is consistent with an internal deletion of about 300 bases[31]. Since the mRNA is itself shorter, we conclude that the deletion originates in a deletion at the DNA level.

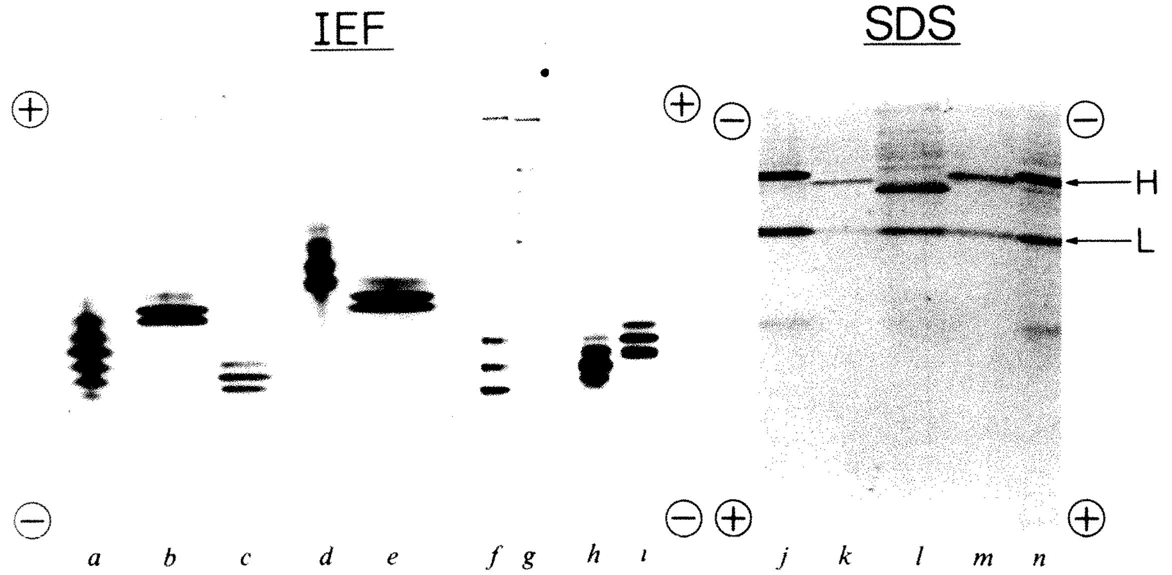


Fig. 3 Comparison of wild-type and mutant immunoglobulins (or Ig chains) by isoelectric focusing[20] (IEF) (*a–i*) or SDS–polyacrylamide slab gel electrophoresis[49] in a 20% acrylamide 0.065% *bis*-acrylamide gel (*j–n*). Igs were reduced by boiling with 2-mercaptoethanol in 1% SDS before applying to the gel. Ig was prepared either by the incorporation of ^{14}C-lysine into secreted protein by cells in culture[20] (*f, g, j–n*), in which case proteins were visualised by autoradiography of the dried gel, or from the sera of tumour-bearing mice[25] (*a–e, h, i*). Typical yields of Ig ml^{-1} of mouse serum were: wild type, 10–20 mg; IFI, 1–2 mg; IF2, 2–4 mg; IF3, 0.7–1.5 mg; IF4, 4–6 mg. For isoelectric focusing the *p*H gradients were: 5-8.5 (*a-e*), 6-8 (*f, g*), or 5-8.5 in 6 M urea (*h, i*). (*b, e, f, n*), wild type; (*a, m*), IF1; (*c, k*), IF3; (*d, j*), IF4; (*g, l*), IF2; (*h*), wild-type H chain; (*i*), IF4 H chain. +, anode; −, cathode. H, L, indicate the position of unlabelled marker wild-type H and L chain.

A frameshift mutant

IF3 was more basic than the wild type (Fig. 2), and its *p*I was not altered by neuraminidase treatment. As in IF1 and IF2, its L chain comigrated with wild-type L chain in SDS–polyacrylamide gels. The H chain had a faster mobility than (Fig. 2) and a different amino acid composition from wild-type H chain[29]. The three C-terminal CNBr fragments of the wild type (H8, H9 and H10, Fig. 4) were not present in IF3 H chain. The six N-terminal CNBr fragments (H1-H6) were unaltered, as judged by their amino acid compositions, the electrophoretic mobilities of fragments H4 and H5, and fingerprints and amino acid compositions of the tryptic peptides of the larger fragments. A new CNBr fragment, IF3-H7′, was isolated[29]. It had approximately the same size as wild-type H7, but a different amino acid composition. This CNBr fragment had no homoserine and was presumably the C-terminal fragment of IF3 H chain. Tryptic peptides corresponding to residues 304-338 from wild type were present in IF3-H7′. Fingerprinting of a tryptic digest of performic acid-oxidised IF3 H chain showed a peptide, IF3H-T28, that was not present in fingerprints of wild-type H chain. This peptide was isolated in very good yield and had the sequence:

Ala-Pro-Gly-Val-His-His-Ser-Thr-Ser-Gln-Gly-Ala-Asp-Gly-Gln-Gly

It was concluded[32] that this peptide corresponds to residues 339 to the C terminus, as the terminal glycine does not represent a point of tryptic, pseudotryptic, or CNBr cleavage. This peptide, plus those isolated for residues 304–338, completely account for the amino acid composition of IF3-H7′. Thus, the sequence of IF3 H chain is identical to the wild type in the first 340 positions. The sequence of the C-terminal 14 residues is not found in wild type, other mutants, or any other immunoglobulins, but it can be related to the sequence of wild type by postulating a -2 frameshift mechanism (Fig. 5). This explanation fits with the published mRNA sequence data and allows a unique sequence of 55 bases to be deduced for wild-type H-chain mRNA. It predicts that UAA (‘ochre’) is the terminator codon in the mutant. This is supported by experiments[33] showing that when IF3 H-chain mRNA was supplemented with UAA-suppressor tRNA in a cell-free system, suppression was detectable, and a new product larger than mutant but smaller than wild-type H-chain was found. No difference in the size of the mRNA was detected by sucrose gradient centrifugation[31]. The mutant is therefore a frameshift mutant due to a deletion of two of the bases coding for Pro-Gln residues at positions 340–341 of the H chain.

A point mutant

IF4 Ig was more acidic than wild type (Fig. 3). The *p*I was not altered by neuraminidase. Isoelectric focusing of partially reduced and carboxymethylated chains showed that the difference was located in the H chain (Fig. 3). No differences were detected between wild-type and IF4 H-chains, by SDS–polyacrylamide gel electrophoresis or amino acid analysis.

The main product of limited peptic proteolysis of wild-type Ig is fragment (Fab′)$_2$[25], a four-chain molecule made up to two intact light chains and two Fd′ fragments (residues 1–232 of the H chain). The (Fab′)$_2$ fragments from wild type and IF4 had identical *p*I, as did the main CNBr fragment (residues 143–303) of the H chains. The only detectable abnormality of IF4 (refs

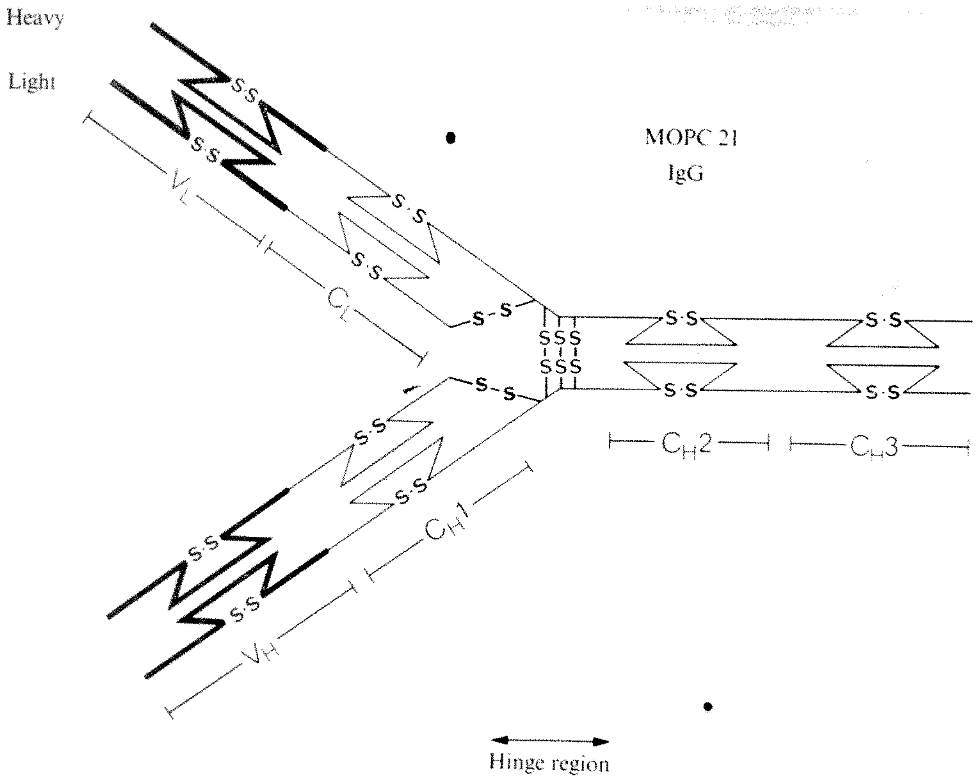


Fig. 1 Diagrammatic representation of the IgG molecule. The pseudosubunits are named and the antigen binding domain (V region) is marked with heavy lines.

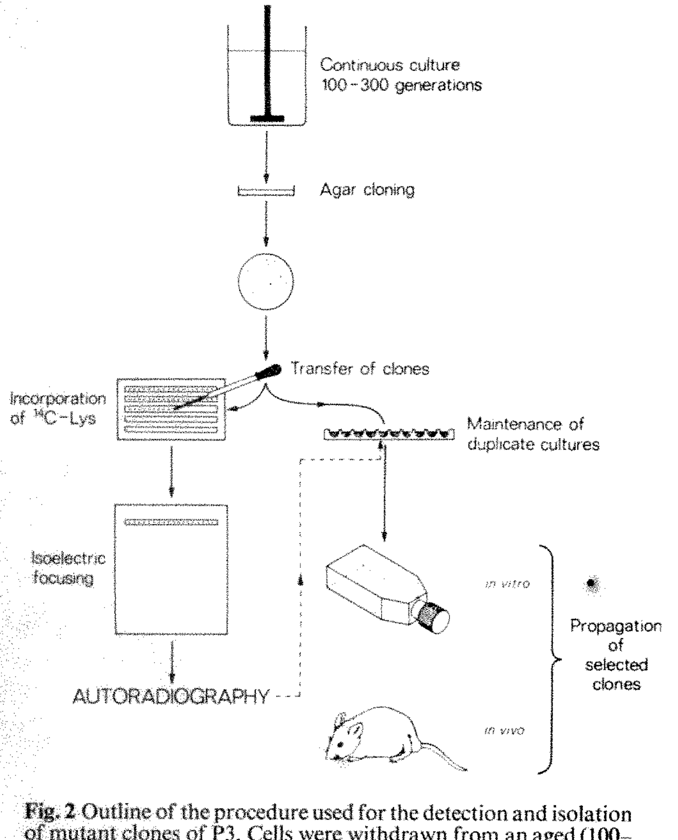


Fig. 2 Outline of the procedure used for the detection and isolation of mutant clones of P3. Cells were withdrawn from an aged (100–300 generations) spinner culture and subcloned in agar. When subclones were visible to the naked eye (12–14 d) they were transferred on to a layer of dialysis membrane on agar growth medium containing ^{14}C-lysine and incubated for 24 h. Replicas of sampled clones were maintained in microculture wells. After incubation the dialysis membrane containing the radiolabelled samples was applied on to thin slabs of polyacrylamide (4.5%) gels and isoelectric focusing was performed. The radioactive immunoglobulin bands were visualised by autoradiography. Further details have been published[20]. Clones whose Ig bands show an altered isoelectric point were recovered from the replicas in microculture wells, grown to mass in suspension, and inoculated into BALB/c mice, in which they grew as solid tumours.

radioactive peptides from peptic and tryptic digestions of labelled wild-type and IF1 H chains[28] showed that the marker peptides from the C_H3 pseudosubunit (see Fig. 1) were missing. Cyanogen bromide fragmentation of IF1 H chain confirmed these findings. The three CNBr fragments of C_H3 (H8, H9, H10) (Fig. 4) were missing. A new CNBr peptide with N-terminal alanine was found, corresponding to the sequence Ala-Lys-Asp-Lys-Val_{357} (ref. 29). Since this peptide had no homoserine, it was presumably the C-terminal fragment of IF1 H chain. The other CNBr fragments were identical to wild type fragments by amino acid analysis and peptide finger-printing techniques. No internal duplication of sequences was detected, so IF1H represents residues 1–357 of the wild-type sequence (Fig. 4), and is 83 residues shorter than the wild-type protein.

The mRNA from this mutant can be translated in cell-free systems. The translation product is smaller than the wild type, indicating that the mRNA itself is altered[31]. A comparison of the size of the mutant and wild-type H chain mRNA by sucrose gradient sedimentation showed that the protein deletion was not due to a corresponding deletion at the mRNA level. The mutation is therefore most probably due to a nonsense mutation in the codon of Ser-358 resulting in premature chain termination. The reason for the alteration in the carbohydrate composition remains unknown. It is possible that this is a secondary effect due to local changes in protein folding. This may lead to a new site for carbohydrate attachment or greater accessibility to sialic acid addition.

An internal deletion

IF2 IgG was more acidic than the wild type counterpart, and its heavy chain was smaller (Fig. 3). Neuraminidase treatment did not affect the *p*I (ref. 30). Fingerprint studies of ^{35}S-cystine and ^{14}C-lysine, ^{14}C-arginine labelled IF2 H chain showed that the C_H1 (see Fig. 1) peptides were missing and that the hinge region peptide was altered. CNBr digestion of IF2 H chain produced a new fragment, H56, in place of H5 and H6 (Fig. 3). A sequentator analysis of H56 gave the N terminal sequence:

108 120 215
Asp-Tyr-Trp-Gly-Gln-Gly-Thr-Ser-Val-Thr-Val-Ser-Ser-Val-Pro-Arg-Asp-CMC....

review article

Molecular analysis of spontaneous somatic mutants

Kayode Adetugbo*, César Milstein & David S. Secher†

Eukaryotic structural gene mutations occurring spontaneously in a mouse myeloma cell line offer the opportunity to study somatic mutation in animal cells at the molecular level. Studies on the myeloma protein and on mRNA have enabled us to characterise four such mutants representing four different mutation mechanisms. The results may have some bearing on the origin of antibody diversity.

THE nature and rate of mutation in somatic cells is of considerable interest in connection with a wide range of biological phenomena including carcinogenesis[1], immunology[2] and ageing[3]. Many cases of somatic mutations in animals are known and several have been extensively studied at the phenotypic level (for example mosaicism of fleece type[4]). In no case, however, has the phenotypic variation been unequivocally shown to be due to a mutation in a given structural gene. In some cases, for example certain haemoglobinopathies[5], non-inherited alterations of haemoglobin have been reported. But these could equally be new spontaneous mutants of the germ line cells. Studies of somatic variants in animals are limited by the death of the individual. For this reason most of the efforts to characterise somatic mutants in eukaryotes have been made on cells in culture. This was made possible by developments in cloning techniques[6] and a considerable number of examples of clonal variants have now been reported[7,8].

It was originally suggested[9,10] that variants in tissue culture were of epigenetic origin, that is heritable variations (as observed in differentiation) not involving alterations in DNA sequence. More recent reviewers, however[7,11], have concluded that many of the described variants are indeed genetic. But the localisation of these mutations to specific structural genes remains incomplete. The presence of altered proteins in variant clones provides preliminary evidence for structural gene mutations. Alterations in enzyme activity[12-14], immunoreactivity[13,15,16], temperature sensitivity[12,17-19] and electrophoretic properties[20] have been taken as evidence of structural gene mutations. Chemically-induced mutant cells with no hypoxanthine guanine phosphoribosyltransferase activity, for example, have been shown to contain material that cross reacts immunologically with the enzyme and is presumably inactive protein; this material comigrates with wild-type enzyme in SDS–polyacrylamide gels[13,15]. Hochman *et al.*[21] have pruified the regulatory subunit of cyclic AMP-dependent protein kinase in mutants of mouse lymphoma cells; the mutant protein has altered temperature sensitivity and enzymatic properties. Such mutants are presumably due to structural changes in the protein and may well be the result of mutations in the structural gene. But these methods of analysis do not exclude changes in post-translational modifications such as carbohydrate addition, phosphorylation, processing of precursor molecules, and so on. These often modify physicochemical and biological properties of protein molecules.

Amino acid sequence analysis of the altered proteins, to allow a more exhaustive characterisation of mutants, is hampered by the difficulty in obtaining enough material. This can be, at least partially, overcome by the use of radioactive tracer techniques. This approach was successfully used for the preliminary characterisation of structural mutants of immunoglobulin genes in myeloma cells in culture[16,22]. Further characterisation of such mutants, including complete amino acid sequence analysis, is possible. Preparation of large quantities of mutant protein is simple when the cells are grown as tumours in mice since the myeloma proteins accumulate in the serum.

Spontaneous structural gene mutants

The mouse plasmacytoma MOPC 21 is an experimentally-induced tumour which secretes an immunoglobulin (Ig) consisting of γ1 heavy chains and kappa light chains [IgG1(κ)] (ref. 23). Both chains have been fully sequenced and the arrangement of disulphide bridges elucidated[22,24,25]. The light (L) and heavy (H) chains are made up of pseudosubunits of ~ 110 residues, each containing a disulphide loop (Fig. 1. For further details of Ig structure and genetics see ref. 26). A line adapted to permanent growth in tissue culture has been derived and is known as P3 (ref. 27). This line has been repeatedly cloned and individual subclones have been screened for the presence of isoelectric focusing variants of the secreted immunoglobulin. The procedure (outlined in Fig. 2) allowed the analysis of a random sample of 7,000 subclones.* Five mutant clones, secreting immunoglobulins of altered isoelectric points were detected, but one was lost. Figure 2 shows the other four, which we named IF1, IF2, IF3 and IF4. Initial sequence studies were at the radioactive level with material prepared in tissue culture[28]. More conventional methods[29] required milligram quantities of material which was prepared by injecting the mutant cells into BALB/c mice. Tumours were generally obtained directly, but sometimes irradiated mice (400 rad) were used during the early passages. The yield of mutant proteins in the serum of the tumour-bearing animals was lower than in the wild type (Fig. 3).

A nonsense mutant

IF1 had a higher *p*I than the wild-type protein and its H chain a lower molecular weight than wild type by SDS–polyacrylamide gel electrophoresis (Fig. 3). Neuraminidase treatment, to remove specifically sialic acid, followed by isoelectric focusing, produced a further shift in IF1, but no effect on wild type[30]. Thus, in addition to primary sequence differences (see below), IF1 Ig had sialic acid, whereas wild-type Ig did not. A comparison of the

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In an attempt to understand better the molecular nature of toxin synthesis, So *et al.* have recently cloned the genetic determinant of ST toxin (*J. Bact.* **128**, 463; 1976). Using the well established techniques of *in vitro* DNA recombinant research, they were able to clone a 5.7 megadalton *Eco*RI-derived DNA fragment determining toxin production in plasmid pSC101 which carries a tetracycline resistance marker, and in plasmid RSF2124 which is an ampicillin-resistant ColE1 derivative. This latter plasmid is present in 20–30 copies per cell and continues replication after chloramphenicol treatment of cells containing it, allowing isolation of large quantities of cloned DNA. Thus the tools are now available to investigate the properties of the toxin and its coding DNA sequence more thoroughly. The cloned sequence or a part thereof can be used as a 'probe' to detect rapidly the presence of ST toxin DNA and related sequences elsewhere. It should also now be possible to study transcription and translation of the cloned DNA, either in *E. coli* 'minicells' or *in vitro*.

This is the first published report of the cloning of a genetic determinant of bacterial pathogenicity, and it is interesting to note how these experiments conform to the various regulations and guidelines intended to control *in vitro* DNA recombinant research. In the USA, the NIH guidelines fairly explicitly categorise such experiments as requiring low containment (P2, EK1), since ST toxin is classified as of low pathogenicity. Such containment conditions at least would normally be used for handling the original toxin-producing strains. Experiments involving DNA of moderate pathogenicity, for example that isolated from *Vibrio cholerae*, would in addition require a disabled vector (P2, EK2), whereas DNA from organisms of high pathogenicity should not be cloned at present. In Britain, the situation is less clear, as experimental protocols and details of available facilities now have to be submitted for consideration by the Genetic Manipulation Advisory Group (GMAG), although the report of the working party on genetic manipulation indicates that cloning of DNA from "bacteria specifying toxins virulent to man" should only proceed with a disabled vector and under the highest physical containment (category IV), whereas cloning of "bacterial DNA non-pathogenic to man" may be carried out under minimal containment conditions.

Much then depends on whether ST toxin is considered virulent to man. It might be good practice for the newly constituted GMAG to consider and decide the categorisation for the So *et al.* experiments! The authors of the paper discuss the possible hazards, implications and benefits of their work. They point out that the ST-toxin determinant was found naturally in *E. coli* as part of a common type of conjugative plasmid which often also contains drug resistance genes. Tetracycline resistance and ampicillin resistance, which are present in the cloning vectors, are often present in toxigenic *E. coli* strains. Therefore no new combinations of drug resistance and toxin production had been produced by the cloning experiments. In addition, the authors were aware that ST toxin production in *E. coli* K12 (the strain used in the laboratory) was unable to cause disease symptoms. Even production of both toxins is not by itself sufficient for a strain to be pathogenic. A host of other, as yet poorly-defined characteristics are required: some concern the properties of the bacterial cell surface and at least one, the K antigen, is plasmid encoded.

Taking this into account So *et al.* felt that the new toxin-producing plasmids, if properly contained, present less of a hazard than the original plasmid in its original strain. It is now known than many drug-resistance genes are readily transposable from replicon to replicon, allowing new combinations of plasmid-borne drug resistance to arise at high frequency. It seems likely that many other plasmid determinants including toxin production will also turn out to be transposable; therefore it looks as though any gene combination that can be made from *E. coli* plasmids by the techniques of *in vitro* DNA recombinant research can occur fairly readily in nature, and if suitable conditions (those provided by the indiscriminate use of antibiotics for example) are available, then hazardous combinations of drug resistance and genes for pathogenicity can be selected and may become a serious problem for the medical and veterinary profession.

Williams Smith has also shown (*J. gen. Microbiol.* **47**, 153; 1967; *J. gen. Microbiol.* **83**, 95; 1974) that production of α-haemolysin and a toxin known as Vir is plasmid specified and associated with pathogenicity of invasive *Escherichia coli* strains. Similarly the presence of plasmids determining colicin V production seems to enhance pathogenicity, apparently by increasing survival of bacteria in blood and the peritoneum. If this effect is the result of colicin V production, then one has the intriguing example of a colicin protein, which is normally only considered to be active in killing *E. coli*, being involved in pathogenicity.

Studies of bacterial toxins are of interest not only to the bacteriologist. Both cholera and diphtheria toxin have already proved invaluable tools to molecular biologists studying eukaryotes because of their highly specific hormone-like interactions both with the cell surface and with their targets within the cell. Cholera toxin has been used as a tool to investigate membrane receptors, as a model for the study of hormone action and as a cyclic AMP probe (for example Finkelstein, *Microbiology-1975*, American Society for Microbiology, **237**, 1975). Similarly diphtheria toxin has been used to block translation of mRNA into protein by enzymatically depleting elongation factor 2 (EF-2) in eukaryote cells. There is also speculative material for the molecular evolutionist: are these toxins of eukaryote origin or can their interactions with the eukaryotic cell throw light on the relationships between eukaryotes and prokaryotes? □

Erratum

In the article 'Diamond platelets are interstitial carbon' (*News and Views* **265**, 208; 1976) line 5, paragraph 3 should read ". . . concentrations up to 1%" and not "up to 12%", and line 13, paragraph 5 (page 209) should read "No more than 3%" and not "No more than 5%".

A hundred years ago

THE electric light is becoming common in Paris in connection with works that have to be carried on during the night. A large lamp fed by a six-horse power has been established in the Avenue de l'Opéra, and others are employed in the Trocadero in connection with the building of the Exhibition Palace. The gramme machine and screw regulator are employed.

THE INTRA-MERCURIAL PLANET.—In M. Leverrier's last communication to the Paris Academy on the planet assumed to exist within the orbit of Mercury, it was mentioned that, with the elements adopted, or very similar ones, a solar conjunction would occur on March 22, and a transit over the sun's disc was possible, though uncertain. A close examination of the disc is therefore to be recommended on March 22 and 23, and there is reason to believe that observers in widely-differing longitudes are prepared to undertake it. If no transit should then occur, eight or nine years may elapse before one is possible at the spring node.

From *Nature* **15**, January 25, 282, 285; 1877

Regulating gene expression in differentiation

from Carol K. Klukas

CONSIDERING the number of different cell types which exist in any mature eukaryotic organism, and the number of individual differentiation events which must have occurred in precisely ordered sequence in the course of its development one is left in no doubt that the system which so precisely controls genome expression in eukaryotes must be incredibly complex.

Research into many different aspects of the control system is currently in progress in many laboratories, and a recent paper from Strom and Dorfman (*Proc. natn. Acad. Sci. U.S.A.* **73,** 3428; 1976) offers new evidence in support of the theory first put forward by Britten and Davidson, that the intermediate repetitive sequences in the genome may have a crucial role in regulating gene expression.

An early theory of the control of eukaryotic gene expression which proposed that appropriate structural genes are singled out and amplified during differentiation has generally proved to be untrue. Gene titration experiments have indicated only one or at most a few copies of any one structural gene, even in cell types where the protein that the gene specifies makes up a large proportion of the total protein synthesised. Research has therefore concentrated on the investigation of possible transcriptional controls, such as the interaction of non-histone proteins and hormones with chromatin, and the sequence organisation of the DNA itself. In 1969 Britten and Davidson (*Science* **165,** 349; 1969) first suggested how, theoretically, intermediate repetitive sequences might control gene expression, and more recently (Davidson *et al. Cell* **4,** 217; 1975) they have found that 80–100% of the mRNA molecules present in sea urchin embryos are transcribed from single copy DNA sequences which are adjacent to interspersed repetitive sequences in the genome. This finding does indeed suggest the regulatory importance of these sequences.

In a recent issue of *Proc. natn. Acad. Sci. U.S.A.* (**73,** 3428–3432; 1976) Strom and Dorfman offer new data which more directly supports this theory and which suggests a specific mechanism by which such intermediate repetitive sequences in the DNA may control differentiation. They use the chick limb bud system in which chick stage 24 limb bud mesenchymal cells are dissociated and cultured *in vitro* at high density. Once the cell density becomes greater than confluency these cells differentiate into cartilage unless the thymidine analogue 5-bromo-2′-deoxyuridine (BUdR) is present at a concentration of 32 mM during the initial 48 h of culture in which case differentiation is irreversibly inhibited. In this study DNA was prepared from early cultures of these limb bud cells grown in ^{3}H-BUdR. The reassociation rate of this DNA was then measured after shearing and in the presence of a 100-fold excess of DNA prepared from cells at various stages of cartilage differentiation or from liver tissue or from whole chick embryos. These experiments show that sternal cartilage DNA and 8-day cultured limb bud cartilage DNA drive the reassociation reaction about twice as fast as does liver DNA or undifferentiated limb bud DNA or DNA from 8-day limb bud cultures blocked from differentiation by BUdR treatment. Furthermore, by analysing the reassociation kinetics of these reactions Strom and Dorfman determine that the total percentage of the tritium which reannealed in the moderately repetitive fraction was lower in liver DNA (20%) than in sternal cartilage DNA (30%), 8-day limb bud culture DNA (30%), or in total embryonic DNA (33%). These data suggest that in cells differentiated for cartilage production a certain set of intermediately repeated sequences is amplified, whereas in undifferentiated cells or in cells differentiated for other functions, this particular complement of intermediate repetitive sequences is not amplified.

Does this mean that the determinative event in the differentiation of cartilage cells might be the amplification of cartilage-specific sequences initially present in multiple copies? Several facts about this system suggest this to be true. First it must be noted that during the first 48 h of culture during which the differentiation process can be blocked by the presence of BUdR, very little cell division occurs. After 48 h the differentiation process is no longer sensitive to BUdR. Since it is known that the half life of ^{3}H-BUdR in substituted DNA is 11 times shorter than ^{3}H-TdR, it stands to reason that the mechanism of BUdR inhibition of differentiation is that although the amplification of cartilage-specific intermediate repetitive DNA sequences does occur in these cultures during the first 48 h, the amplification is accompanied by and followed by rapid degradation of the sequences (because they contain BUdR) so that the cell does not receive the proper differentiation signal.

Similar BUdR sensitivity is exhibited by differentiation processes in various other chick cell types and in other tissues of diverse eukaryotic organisms. Could it be that this proposed amplification event is a generalised feature of the cell differentiation process and that it is somehow involved in the regulation of genome expression? A positive answer to this question is certainly possible, but regardless of the outcome, further reports of research on this phenomenon are sure to be forthcoming soon and certainly promise to be exciting. ☐

Plasmids and pathogenicity

from David Sherratt

ESCHERICHIA COLI has often been considered a harmless inhabitant both of the large intestine of man and animals and of molecular biology laboratories. However in the past ten years it has become increasingly clear that some strains of *Escherichia coli* can be pathogenic. Enteropathogenic strains in the gut cause diarrhoeal diseases and invasive strains cause bacteraemia and urinary infections. Diarrhoeal disease resulting from enteropathogenic *E. coli* may well be one of the leading causes of death of young children in developing countries (see Sack, *A. Rev. Microbiol.* **39,** 333; 1975), and enteropathogenic *E. coli* cause considerable losses in intensively reared livestock.

Enteropathogenic *E. coli* strains produce either or both of two types of protein exotoxin, one heat stable (ST) the other heat labile (LT). The latter resembles cholera toxin and produces a similar but comparatively short-lived massive salt and water secretion from the mucosal cells of the small intestine —hence the diarrhoea. The similar effects of ST toxin are even more short-lived and the biochemical basis for its action at the cellular level is unknown. H. Williams Smith (*J. gen. Microbiol.* **52,** 319; 1968) was the first to show that toxin production in *E. coli* is transmissible by conjugation, and this led to the finding that the toxins are plasmid specified, often by the same plasmid.

Little is known about the genetic determinants of toxins in other microorganisms, partly because little is known of their molecular biology. The genes for cholera toxin seem to be chromosomally located and the structural genes for diphtheria toxin are known to be located on a lysogenic bacteriophage. In addition, the genes responsible for causing crown gall tumour in plants seem to reside on a plasmid present in the causal microorganism *Agrobacterium tumefaciens.*

positive and negative RNA strands, that hybridise into double-stranded hair-pin forms.

Rabies is another rhabdovirus which, unlike VSV, spontaneously gives rise to persistent infections in cell culture without the addition of DI particles (Wiktor & Clark, *Infect. Immun.* **6**, 983; 1972). However, DI particles are soon found in the infected cultures (Crick & Brown, *J. gen. Virol.* **22**, 147; 1974) and Holland *et al.* show in their paper (*op. cit.*) that even after cloning, rabies virus quickly generates new DI particles. The rate of generation of defective virions may therefore determine whether a particular virus or strain gives rise to acute lytic or persistent infection.

Other virologists (Stanners & Goldberg, *J. gen. Virol.* **29**, 281; 1975; Youngner *et al.*, *J. Virol.* **19**, 90; 1976) have shown that temperature-sensitive VSV mutants can give rise to persistent infections and have emphasised the possible role of conditonal mutants in viral disease. The champions of DI particles point out that TS viruses generate DI particles more readily, but a curious finding is that persistent infections initiated by wild type plus DI particles also generate a high frequency of TS particles among the mature progeny. Furthermore, VSV and many arboviruses replicate in insect cells without cytopathic effects and therefore the wild type readily establishes persistent infections in these cells (which are probably their ancestral as well as present hosts). Yet one finds, as Mudd *et al* (*J. gen. Virol.* **20**, 341; 1973) have shown, that many of the progeny replicated in insect cells become temperature sensitive, and will not grow at 37 °C. Perhaps the virus strains that are virulent in warm-blooded animals represent heat-resistant variants rather than true 'wild types'. Clearly, there is plenty to think about and investigate in persistent viral infections and virus-host interactions without worrying about DNA copies.

Having returned to the question of unusual proviruses, however, it is noteworthy that Zhdanov's and Iinuma's papers indicate that reverse transcriptase was associated with the viruses in their persistent infections, and one wonders whether retroviruses and other viruses might interact genetically in mixed infections. Since many commonly used cell lines are persistently infected with C-type viruses (which can 'persist' as host genes), this question merits investigation. Incidentally, strange transcripts of retrovirus genes themselves have recently come to light. Retroviruses are classified as 'positive strand' RNA viruses as their genomic RNA has the same sense as mRNA. As they replicate by transcription of a DNA provirus one would not expect to find negative strand viral RNA as a replicative intermediate. Recently, however, Stavnezer *et al* (*J Virol.* **20**, 343; 1976) have detected by hybridisation to virion RNA the presence of negative RNA strands in the nuclei and cytoplasm of infected cells, representing up to 30% of the genome in avian sarcoma virus infection, and 45% with murine mammary tumour virus. Previous attempts to detect negative strand RNA may have failed because the RNA is already duplexed with positive strands. The function, if any, of the negative strand RNA is not yet clear. □

Cosmic X- and γ-ray line radiation

from Andrew Fabian

X- and γ-ray astronomers have been observing an increasing number of spectral lines over the past year or so. Most of the X-ray reports have come from two groups. the OSO 8 group at the Goddard Space Flight Center; and the Ariel V group at the Mullard Space Science Laboratory of University College London Line radiation at about 7 keV, presumably due to iron (which is by far the most abundant element emitting at this energy), has been detected to date in one binary X-ray source, Cyg X-3; several supernova remnants (for example, Cas A, Tycho and G287.8-0 5) and several clusters of galaxies (the Perseus cluster for example). All of these results (reported in the *Astrophysical Journal* and the *Monthly Notices of the Royal Astronomical Society*) derive from satellite-borne proportional counters, which have good sensitivity but poor energy resolution. The few high resolution crystal spectrometers carried into orbit are not yet sensitive enough to resolve such features.

The mere detection of line radiation from clusters of galaxies strongly favours a thermal origin for the X-ray emission, and indicates that the hot gas responsible cannot be wholly primordial. Presumably some heavy element enrichment has taken place. Most of the heavy elements are generally considered to be made in supernova explosions, and X-ray spectroscopy of their remnants may offer a direct means of checking that hypothesis. The preliminary results indicate that iron need not be particularly overabundant, but that result is, of course, dependent upon the structure of the remnant, and the proportion of ejecta to swept up material currently radiating X rays. Cyg X-3 is a particularly enigmatic binary source, for it emits throughout the spectrum from radio waves to γ rays. Indeed there is no real proof that it is a binary source, for any companion is only inferred indirectly from the observations. Whereas the iron line radiation from the clusters of galaxies and supernova remnants is probably due to transitions in Fe XXV and Fe XXVI, the feature in Cyg X-3 may be due to fluorescence of 'cold' iron. A slight improvement in sensitivity and resolution should decide this point, since the 'hot' and 'cold' features differ significantly in energy Variations in intensity over the 4.8-h cycle of Cyg X-3, and any future detection of periodic Doppler shifts in the line energy will undoubtedly be important in sorting out the true nature of this system. This work is only in its infancy, and the detection and resolution of more X-ray lines due to iron and the lighter abundant elements will be a worthwhile task.

γ-ray lines observed at energies above 0.511 MeV (the electron–positron annihilation line) are presumably due to nuclear transitions. Such radiation has been predicted to occur due to radio activity in supernovae and the interaction of low energy cosmic rays with interstellar material, and has been observed in intense flares in the Sun. A group at Rice University, Texas, have recently published a detailed account (*Astr. J.* **210**, 631–641; 1976) of their earlier reported detection of nuclear γ-ray lines in Centaurus A. At ~5 Mpc, it is the nearest bright radio galaxy, and contains a nucleus varying at X- and soft γ-ray energies. R. D. Hall, C. A. Meegan, G. D. Walraven, F T. Djuth and R. C. Haymes claim the detection of features at 1.6 MeV and 4.5 MeV, both 3.3σ above the neighbouring continuum. Since they have about 40 energy channels in their balloon-borne scintillation counter, and they could have seen lines in any of these, this result is significant only at the ~2% level. Similar results on the galactic centre region have earlier been reported by the same group. Centaurus A is a reasonable place in which to expect plenty of low energy cosmic rays, and the absorption observed at X-ray wavelengths indicates the presence of a considerable amount of interstellar gas. It will be exciting if the intensity of the γ-ray line activity is found to vary in a similar fashion (but out of phase?) to the X- and γ-ray continuum. The authors note that the total low-energy γ-ray luminosity of Centaurus A is about 10^{44} erg s^{-1}. Since this is about ten times its radio luminosity, perhaps we had all better start referring to it as the nearest γ-ray galaxy. □

Restating the self-thinning rule

from Peter D. Moore

MORTALITY amongst young organisms is one of the basic observations which led Darwin to propound his theory of natural selection. Often this mortality results from the competitive stresses which are derived from a high density of other members of the same species. Among plants the process is often referred to as self thinning. The nature of this process was analysed by Yoda *et al.* (*J. Biol. Osaka City Univ.* **14**, 107; 1963) in pure even-aged populations of plants under density stress. Their most important finding was that if one plots the log of mean individual plant weight against the log of surviving plant density one obtains a straight line with a slope of $-3/2$.

The self-thinning rule which emerges from these observations can be summarised by the formula,

$$W = Kp^{-3/2}$$

where W = mean plant weight and p = density.

The rule was examined by White and Harper (*J. Ecol.* **58**, 467; 1970), who provided new experimental data and who also analysed a body of published data most of which agreed with the observations of Yoda *et al.* Variations in nutrient availability had no influence upon the slope of the line, but did affect its position on the axes. Fertility resulted in a higher degree of thinning. On examining published data concerning light intensity variations and thinning in *Helianthus annuus*, however, they found that decreasing light intensity was correlated with less steep slopes in the log plant weight/log density lines.

Kays and Harper (*J. Ecol.* **62**, 97; 1974) have since experimented with the rye grass, *Lolium perenne* and have tested the effect of light intensity upon both individual (genet) and tiller density and weight. At high light intensities genet weight and density obeyed the 3/2 thinning law, but at the low light levels the slope fell to 1. On this basis they suggested that it is the reduced light intensity within a sward which causes the stress, resulting in mortality.

In this issue of *Nature* (page 330), Westoby re-examines the 3/2 self-thinning law in the context of work on the influence of light on leaf morphology. It is known that leaves produced under low light intensity have a larger area, but not normally a greater weight, than those produced in high light levels. Area : weight ratios are therefore raised at low light. This being so, Westoby has re-examined the original *Helianthus annuus* data analysed by White and Harper, but has plotted mean leaf area on a log scale rather than log mean plant weight. The result is a line with a 3/2 slope which is independent of the light intensity at which the data was obtained. Thus he reformulates the self-thinning rule as

$$L = Kp^{-3/2}$$

where L = leaf area. Not only is this formula of more general application than the original statement of the rule, it also permits one to predict mortality rates given information on the changes in leaf area index (leaf area : ground area) of a population in time. Such information is already available for many crop and forage species, hence one can work from this data to a better understanding of their population dynamics under density stress. □

Persistent infections without proviral DNA

from Robin Weiss

IN 1975, Zhdanov (*Nature* **256**, 471) and Simpson and Iinuma (*Proc. natn. Acad. Sci. U.S.A.* **72**, 3230) claimed to have found integrated DNA copies of RNA viruses such as sindbis, measles and respiratory syncytial viruses in the cells of persistently infected cultures. They suggested that the formation of DNA 'proviruses' might lead to the persistant state. Recently, John Holland and associates (Holland *et al.*, *J. gen. Virol.*, **33**, 193; 1976) have concluded from a series of meticulous experiments on long-term persistent cultures of BHK-21 cells infected with vesicular stomatitis virus (VSV) that there is no evidence for 'proviral' DNA copies of the VSV genome in those cultures. Hybridisation tests capable of detecting 1 viral genome per 40 cells (or 1/40 of a genome per cell) gave no indication of sequences homologous to viral RNA in the DNA of persistently infected cells, and DNA transfection attempts also failed to reveal any biological activity. Transfection with DNA was also negative in a variety of other cultures persistently infected with rabies, influenza, measles, mumps or lymphocytic choriomeningitis viruses. Thus it is clear that the formation of proviral DNA is by no means a prerequisite for the establishment of persistent infections with negative strand RNA viruses.

Holland and Villarreal originally established the persistently infected VSV carrier culture (*Proc. natn. Acad. Sci. U.S.A.* **71**, 2956; 1974) by using a temperature-sensitive mutant (*ts*G31) together with an excess of defective interfering (DI) particles. Nucleocapsids of the DI particles persist in the carrier cultures and presumably interfere with complete particle replication. Nevertheless, most cells in the carrier cultures continue to synthesise viral RNA (Villarreal and Holland, *J. gen. Virol.* **33**, 213; 1976) and proteins even after more than 2 years in culture. Cell cloning can, however, yield 'cured' cell populations lacking all evidence of infection.

Holland's group's studies support the view put forward by Huang and Baltimore in 1970 (*Nature* **226**, 325–327) that defective interfering particles have a role in viral disease processes. Doyle and Holland (*Proc. natn. Acad. Sci. U.S.A.* **70**, 2105; 1973) had previously shown that the addition of excess DI particles to biologically active VSV protected mice against early lethal intracerebral infection. But DI particles might lead to 'slow' persistent infection, as opposed to acute infection, *in vivo* too, and with some viruses (measles, for example) and some hosts (such as man) the consequences to the host may be far more dire than acute infection. Since DI particles depend on non-defective virus for replication, but then replicate to excess and interfere with the non-defective virus, cyclic production of mature and DI virus may ensue, as Palma and Huang (*J. infect. Dis.* **129**, 402; 1974) have shown for VSV when fresh cells are added to infected cultures. The mechanism of interference by DI particles is not clear, but they may compete for polymerase. DI particles typically contain a part of the viral genome as the usual negative strand RNA, although Lazzarini *et al.* (*J. molec. Biol.* **97**, 289; 1975) have described interesting covalently-linked being very brightly marked while the female, presumed to be the fish depicted, is drabber. Such small fish were and are usually cooked whole, and form an essential part of the modern *Zuppa di Pesce*.

(8) Lesser spotted dogfish: *Scyliorhinus canicula*. This is the commonest small shark of the Mediterranean Sea and is taken by net or line on muddy and sandy ground in shallow waters. The flesh is unpleasant when fresh and fishermen often immerse the skinned bodies in seawater to achieve an acceptably flavoured fish (the taint is caused by the presence of a high concentration of urea in the fish's blood).

(9) Sea bream: *Diplodus sargus*. The artist has accurately depicted the dark markings near the tail and under the pectoral fin which distinguish this species from other sea breams.

(10) Red gurnard: *Aspitrigla cuculus*. This is a famous delicately flavoured gourmet fish. The artist has not illustrated the separate 'sensory' rays of the pectoral fin which are usually seen in the gurnards; perhaps they were collapsed or damaged in this specimen.

(11) This is probably a near relative of number 18, *q.v.*

(12) Sea bass: *Dicentrarchus labrax*. This excellent fish may occur in brackish lakes and estuaries as well as in the sea. According to Pliny, the Romans preferred specimens caught in the rivers (*'At in lupis, in amne capti praeferuntur'*), but things are not what they used to be, and nowadays those taken in the sea are better. Bass patrol rocky shores and pounce on shoals of sand-smelt or prawns. They will also maintain an uncanny watch on a SCUBA diver after the style of barracudas in the Caribbean.

(13) Moray eel: *Muraena helena*. This cunning and dangerous fish is highly esteemed by some gourmets, but others deem it worthy only of the *Zuppa di Pesce*. The skin is thick and slimy, but lacks scales. The artist has convincingly depicted the camouflage mottling and the gill opening just behind the mouth.

(14) This is in some ways the most interesting specimen in the mosaic, from the point of view of the marine biologist. It shows a neogastropod whelk having a long grooved siphon, the mark of a hunting snail. The length of this siphon proves the animal to be *Murex brandaris*, one of the snails used by the ancients in the manufacture of the dye Tyrian Purple, used for colouring imperial robes and other garments and furnishings. Whether the specimen illustrated was destined for the table or the dye factory is open to speculation; this species is, under the colloquial name of *murice* (in various parts of Italy) or *sconciglio* (around Naples) still eaten. The modern Neapolitans use the expression 'ugly as a sconciglio' as an insult.

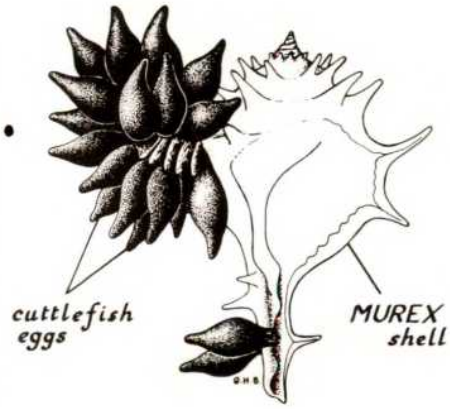


Fig. 2

But this individual is especially interesting because it appears to show around the aperture of the shell a circle of flask-shaped excrescences. These hide the true spines of the shell, which are in any case slender and distinctive in this species, and it appears that they are the eggs of the cuttlefish *Sepia officinalis*. Such eggs are not infrequently found attached to *Murex brandaris* shells, and a drawing of such an association found on the island of Ischia (close to Naples) is reproduced here for comparison (Fig. 2). It seems that the artist may have selected for his sketches just such an individual, perhaps because the unadorned animal was even at that epoch regarded as ugly and uninteresting.

(15) A blenney, of which several species occur in the Mediterranean, in the genus *Blennius*. It is possible but not likely that it is instead a goby. Gobies were not thought highly of as table-fish, although a dissenting Venetian view was recorded by Martial:

> 'At Venice, famed for dainty dishes,
> The Gobies rank the first of fishes.'

(from *Mediterranean Seafood*, Alan Davidson, Penguin Books, 1972)

(16) Striped bream: *Lithognathus mormyrus*. The tail of this species is usually found to be forked (as shown in the bream number 9) but the artist has here illustrated it in a collapsed state.

(17) Smooth hound: *Mustelus mustelus*. This small shark differs from the Mediterranean species of dogfishes (see number 8) by the forward position of the first dorsal fin. In the smooth hound this fin lies above the pectoral fins (more or less), while in the dogfishes it is more to the rear. The artist has shown this feature of shark classification extremely well.

(18) Scorpion fish: *Scorpaena porcus* or *S. scrofa*. There are ten species of scorpion fish in the Mediterranean Sea, and it cannot be certain that the fish portrayed in the mosaic is one of the common ones. They are all bottom-living fish, hunting by twilight, seizing quite large prey with great speed, using the wide powerful mouth. So committed are they to life on the bottom of the sea that the scorpion fishes have lost the swim bladder characteristic of most other bony fish. The dorsal spines can inflict a painful wound if the fish is carelessly handled, and this fact, together with their generally small size, has meant that scorpion fish are utilised chiefly as a flamboyant ingredient in the Neapolitan *Zuppa di Pesce*. The fish in the mosaic was evidently sketched out of water, because the head processes (which normally stand up in water) are not illustrated.

(19) Shrimp: *Crangon vulgaris*. The artist has spread the peraeopods (walking legs) guilefully in order to illustrate all 18, but the chelae (nippers) are not shown so convincingly. This shrimp is usually found on muddy sand in shallow water, and it is drab, small and poor eating. One wonders why the artist chose to include *Crangon*, rather than the more exotic delicate-tasting 'scampi' (*Neophrops norvegicus*) or one of the larger prawns. Perhaps the gear to capture these was at that time nonexistent.

(20) Squid: *Loligo vulgaris*. The diamond shape given to this squid by the position and extent of the gliding 'fins' serves to identify this species, sometimes called the long-finned squid. They usually swim very close to the surface of the sea and can be taken with bony fish in fishing nets. Typical specimens have eight short arms and two long ones, but the artist has shown one whch lacks a short arm. Was this an inaccuracy or had this individual lost one?

(21) Two-banded bream: *Diplodus vulgaris*. The artist has shown the shape of a sea-bream well, but the species must remain a tentative indentification. It is not easy to determine whether certain markings on the mosaic are merely accidental damage.

(22) Octopus: *Octopus vulgaris*. This is the 'Polpo' beloved of modern Italians. The artist has accurately depicted both the restless squirming energy of the octopus and the external features needed for an identification to species. The arms are shown to have two rows of suckers, typical of species of the genus *Octopus* (*Eledone* has single rows), and the proportions are of *O. vulgaris*, not the other Mediterranean octopus, *O. macropus*. Large specimens can reach 25 kg in weight, 3 m across the tentacles; not exactly a kraken, but pretty frightening to meet underwater. □

ings are shown on the dorsal surface, although the number is nearly always five As will be argued below, this represents an error of transcription on the part of the illustrator, and probably occurred between the fishmarket (where he must have made his preliminary sketches) and the execution of the mosaic *in situ* The animals are portrayed in the freshly killed state; they were not observed from life in aquaria. This is evident in a number of cases where fins or other excrescences are illustrated in a collapsed state (as occurs in air but not in water).

No marine biologist could fail to appreciate and admire this extraordinary composition, and one wishes that present-day scientists and their illustrators were always so painstaking and accurate.

(1) Electric ray: *Torpedo torpedo*. This animal is characterised by its possession on the upper surface of several (usually five) dark false 'eyespots' (ring-like markings, not true eyes at all). It is remarkable that the artist has depicted nine such markings and it may be surmised that this represents an error of transcription from the artist's notes and sketches to the mural itself. He has mistakenly depicted the ray's real eyes and spiracles (Fig. 1) (exhalant openings for the respiratory stream) to be four extra false 'eyespots'. This is the only major inaccuracy detectable in the whole mural.

(2) Red mullet. *Mullus surmuletus*. This fish is tantalisingly damaged in the mural and the tendril-like jaw processes characteristic of *Mullus* are not shown, and other features of the snout can only be guessed at. Because of this damage, the possibility cannot be ruled out that this fish belongs to the related variety (or separate species, according to one authority) *Mullus barbatus* Both occur in the Mediterranean Sea. They are among the most highly prized table-fish in the world, and are commonly cooked whole, not gutted. (Naples slang for these fishes is *fragaglie*.)

(3) Grey mullet. *Mugil* sp. A beautiful illustration of a beautiful fish. Grey mullets shoal in coastal waters where they especially favour areas of freshwater inflow, and sometimes enter estuaries and lagoons. There are seven species in the Mediterranean Sea, and only an expert can distinguish them, using whole fresh fish. Highly esteemed for their flavour, grey mullets were in classical times thought to be the most gentle and holy fish because they were herbivorous.

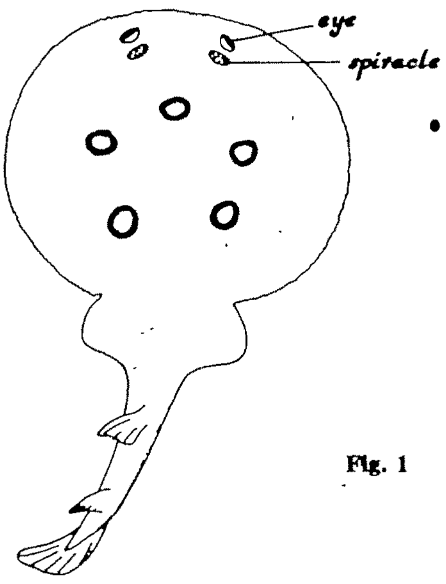


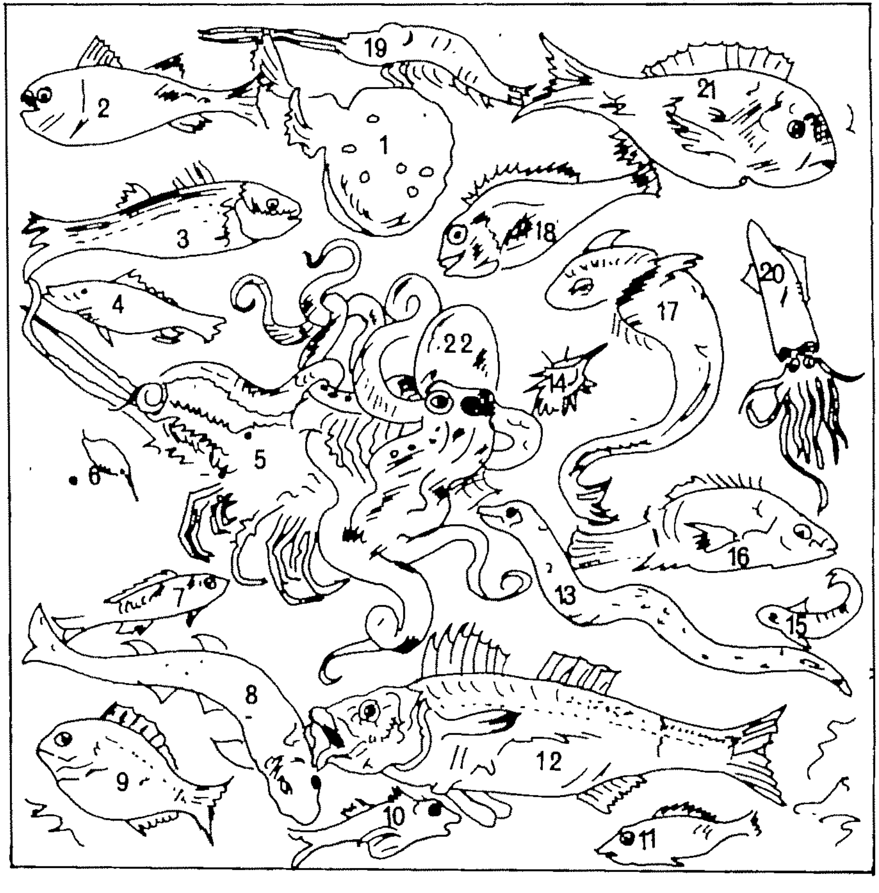
Fig. 1

(4) Wrasse. *Labrus* sp. This genus contains a number of well-established species but it is still difficult to identify the fish in the picture down to species. Wrasses include many common and familiar inhabitants of coastal waters in the eastern Atlantic, many of which are flamboyantly patterned. But the patterns vary with the time of the year and males and females are often quite different. Add to this difficulty the fact that many wrasses change sex as they grow up, and the reader will understand that it is difficult to be precise about this fish. *Labrus merula*, the brown wrasse, comes very close.

(5) Crawfish· *Palinurus vulgaris*, shown in combat (more likely to be imagined than real) with an octopus. It is interesting that the European crawfish is depicted and not the African or Green crawfish, which nowadays often finds its way to the fish markets of Italy. According to a cruel legend recounted by Robert Graves the Emperor Tiberius is said to have ordered a fisherman's death (the unfortunate man had startled the Emperor near the cliff-top in Capri) by the unusual means of having his own catch of crawfish (Suetonius writes instead that it was a mullet) rubbed into his face. He was then thrown from the cliff to the sea and rocks far below. Crawfish are nowadays best caught by SCUBA diving, but two thousand years ago they were probably trapped in baited wicker baskets.

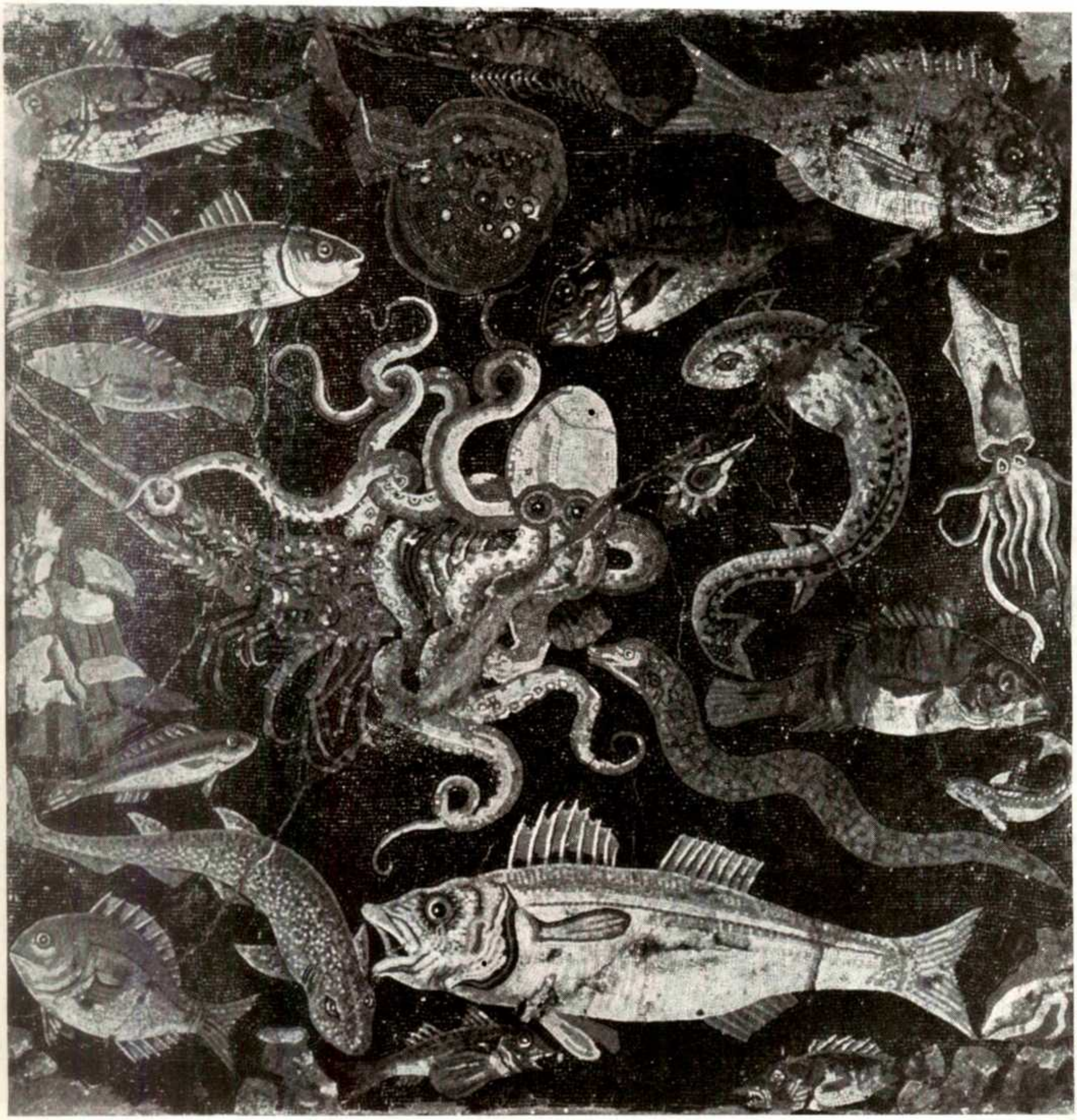
(6) All scientists and illustrators are duffers outside their chosen areas of knowledge, and the author of the mosaic was evidently no ornithologist. This bird is a curious mixture of kingfisher, dunlin and rock pipit. It seems to have been included in the mosaic purely for artistic reasons. Whereas all the marine animals in the composition are represented faithfully and can usually be identified to genus and species, this is a generalised, stylised token bird, Disneyesque in its anonymity.

(7) Rainbow wrasse· *Coris julis*. This is a species which shows pronounced sexual dimorphism, the male



A marine biologist at Pompeii AD 79

from T. E. Thompson



Pompeii Exhibition

THE Pompeii AD 79 exhibition at the Royal Academy contains, in a mural on loan from the Naples Museum (number 120177), a mosaic illustration abut 90 cm square which shows fish and other marine animals of culinary importance from the Bay of Naples. These organisms are portrayed with such vigour and scientific accuracy that marine biologists may well find this mosaic the most interesting and challenging of the exhibits. The artist was a scientific illustrator of rare talent making this composition, originally executed as a domestic decoration, well worth closer inspection.

All the animals shown in the mosaic were primarily edible, with the exceptions of the whelk (number 14 on the diagram) and the curiously artificial bird (6) which was perhaps included simply to fill up a space. The whelk shown belongs to a species of *Murex* which was in classical times the raw material of a great industry surrounding the extraction from its tissues of the dye Tyrian Purple. It does not make good eating, and its modern slang name in Naples (*sconciglio*) is used in an expression of repugnance.

The animals are illustrated with faithful delicacy and accuracy. The only serious error detectable in the mosaic is in the depiction of the electric ray *Torpedo* (number 1 on the diagram), where nine ring-like mark-

news and views

Sea surface temperature and climate

from P. B. Wright

SEA surface temperature (SST) is an important factor in climate. The physical reasons are readily apparent; if relatively cold and unsaturated air blows over the sea, heat and moisture are transferred from the sea into the air, and the rates of transfer depend on the temperature difference. Thus subsequent behaviour of the airstream depends on the temperature of the ocean surface over which it has passed; for example, the latent heat from the additional moisture evaporated into the air may be released into the upper troposphere at some distance downstream, modifying the circulation there. This may in turn influence the circulation even farther afield.

Moreover, SST has high thermal inertia. The sea surface takes a long time to cool down (or warm up) because any change of temperature has to be distributed through the top 100-m layer which is well stirred. Some of the effects of these properties are familiar—maritime climates are more equable and more humid than continental ones, and their seasons are delayed a month or two. It is not surprising therefore to find that SST variations play an important part in causing the climate to differ from year to year. A number of correlations have been discovered over the past few years—between SST near Newfoundland and atmospheric circulation over Europe, SST over the North Pacific and weather over both North America and Europe, and between SST in the Equatorial Pacific and the 'Southern Oscillation', which involves rainfall in Australia and many other regions.

Markham and McLain (see this issue of *Nature*, page 320) have now found a new example, a close relationship between SST in the equatorial Atlantic and rainfall in north-east Brazil. Correlation coefficients reach the remarkably high value of 0.8 in four separate months. It is particularly noteworthy that correlations of 0.7 occur in a predictive formula—between December SST and the following January-to-March rainfall. Moreover they are highly significant, indicating a real physical relationship. The details of the chain of interactions are still far from clear. Warm SST probably favours a modified trade wind pattern, which affects the rainfall. But the physical basis is sufficiently firm, and the relationship sufficiently close (even after allowing for sampling variations), for useful forecasting of the rainfall in this drought-prone region of the globe to be possible. When the physical details become better understood, and the most relevant sea area is pinpointed accurately, improved forecasts should be possible.

The forecasting potential depends essentially on the fact that SSTs in the equatorial Atlantic not only influence the rainfall, but also persist for several months. This is similar to the behaviour of equatorial Pacific SSTs. This raises the following questions. Are there other influences of the Atlantic SST similar to those of the Pacific? Are the two regions in any way coupled? Why do anomalies persist so long, and why do they eventually change? It will be some time before these questions are answered. But another question comes to mind. Are there any other regions where SST anomalies tend to persist for several months or longer? If so, then whatever their cause, we ought to know of their existence, because they would imply a forecasting potential for regions whose climates they influence. This should be simpler to answer; it merely requires a routine statistical analysis of SST data series.

However there are serious problems with data, some of which are mentioned in the paper. It is a tedious and intricate job to transform millions of observations from ships' logs into series of monthly means, checked for errors, for 5° squares over the globe. But the need for reliable homogeneous series cannot be overemphasised. Work is now in progress in the Meteorological Office at Bracknell to devise an objective procedure for identifying errors, which will be a valuable contribution to the task. Nevertheless, as this paper shows, even the data available at present "form a picture that is coherent and of predictive value".

The paper also looks at correlations between equatorial and higher-latitude climates, and suggests a plausible mechanism by which warm South Atlantic sea temperatures could favour a deeper upper-air trough near Newfoundland. This sequence of interactions is the reverse of one put forward by Namias some years ago. The problem of cause and effect is a thorny one. It will not be answered from simple correlation studies like that of Markham and McLain, but these are valuable in providing pointers. Numerical models will have a crucial role; one could impose a persistent SST anomaly and look at the effects in different latitudes. Some such experiments have been made on both the Pacific and the Atlantic, which clearly indicate that equatorial SSTs can greatly influence higher-latitude circulation patterns.

So there is a great deal of further work needed along several parallel lines. The SST data base needs to be improved and made available. The homogeneous series so obtained should be analysed to discover areas where persistence is strongest. These areas should be used in correlation analyses to discover regions for which forecasts are possible, and also in numerical experiments to investigate the physical relationships which underlie the correlations. ☐

IN BRIEF

Scientific freedom

A group of Swedish scientists has recently formed a committee for scientific freedom which is to agitate on behalf of persecuted or harassed scientists in the same way that Amnesty International works for political prisoners. About 100 scientists have so far shown interest in the committee, which is chaired by Professor Ragnar Romanus of Gothenburg. The idea is that physicists will work on behalf of physicists, biologists for biologists, and so on.

The scientists say that their work will not in principle be directed at any particular countries. Several of the committee's members have previously urged the Soviet authorities to grant exit visas to Jewish scientists wanting to emigrate to Israel. They stress however that they do not want to condemn any political system as such, but rather the treatment that various systems mete out to their scientists. At the moment, Argentina is an obvious target for their work.

Hot summer, good news

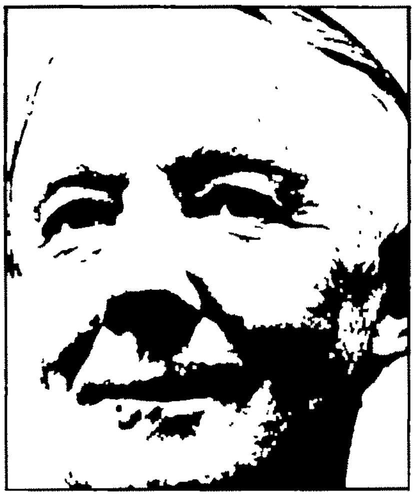
Infant mortality figures for England and Wales in 1976 show a marked improvement on figures for earlier years, and some of this improvement can probably be attributed to the long hot summer. Improvements in obstetric practice and care of newborn infants must also have had an impact, however, in the overall drop in mortality reported by the Office of Population Censuses and Surveys.

Post early neonatal mortality (one week to one year in age) regularly shows a seasonal term, but in 1976 this was exceptionally pronounced, summer deaths being 4.1 per thousand (annual rate) compared with 7.3 for the winter quarter This helped in large part to cause a drop in the whole year's figures by an amount almost equal to the drop for all the previous five years.

Perinatal mortality (28 weeks after cenception to one week after birth) shows a much smaller seasonal term, but there too substantial gains have been recorded. The figure for 1970 was 23.5 per thousand; by 1975 it was down to 19.3 but in 1976 the figure dropped to 17.5.

Infant mortality is now about half the level it was in the early 1950s, but until this past year many people have expressed concern that Britain's performance in reducing the rate did not match that in other developed countries.

Effective science



KENNETH MELLANBY

A FEW years ago politicians were saying that British scientists were about to launch the "white hot technological revolution" which, fanned into flames by the efforts of the civil servants in a newly conceived Ministry of Technology, would revitalise British industry. The revolution, unfortunately, was stifled at birth, and the new Ministry soon disappeared.

Today Britain's scientists are blamed because the growth of industrial productivity is slow or nonexistent. They are castigated because they appear to be unwilling to follow careers in industry, or to direct their research into fields which might ensure economic progress. University courses for undergraduates and graduates are criticised for not imparting the proper skills, and even more for not giving the necessary "motivation". The country's most prestigious committees debate these subjects interminably, but have so far made no suggestions for improvements in education, training or organisation which engender any confidence in academic or commercial circles.

Yet thirty years ago British scientists enjoyed, and may even have deserved, greater prestige. It was generally agreed that they had made many remarkable contributions of a very practical nature to both military and other problems (medicine, public health, transport for instance) during the Second World War. They showed themselves able to tackle and solve difficult and important problems often far removed from those they had previously studied. This they did for the most part with the minimum of expensive and complicated equipment, and often at very little cost to the community. This gave rise to the belief that if Britain increased her scientific establishment many fold, and if its financial support was allowed to grow exponentially (conditions fulfilled during more than twenty post-War years), she would solve many of her social and economic problems.

Unfortunately the results have been disappointing. Today all is not well with both science and industry in Britain and in many other parts of the world, and most attempts to diagnose and cure this malaise have been unsuccessful or have made things worse. Surely the obvious course would be to examine previous successes, and see what can be learned from them. For instance were there any common factors in their education, training and experience which made war-time scientists so adaptable and successful at solving so many of the immediately important problems which faced them and the country?

It is dangerous to generalise, but it is clear that many of those who were most successful in war-time science had previously worked only in university departments or for research councils. Their 'training' had been entirely academic, and, up till then, they had mostly worked in their own way on problems of their own choice. As far as I know, none had ever attended a course in 'management'. In many cases it was the scientists themselves who first became aware of the existence of the problems which they eventually tackled. They often had to convince the authorities, including the expert committee (which existed even then, but fortunately in much smaller numbers than today), that the work was worth doing. And it was often the academic scientists themselves who took the successful initiative in ensuring that their results were put into practice.

In many ways the problems facing the world today are as serious and urgent as those which faced Britain during the war. Can anyone explain why scientists no longer operate as effectively as they did at that time? There are far more trained research workers, and, notwithstanding recent cuts, far more money is spent on science. Surely this is what our committees should be investigating, even if they discover that it is often their own existence which is the barrier to scientific and economic progress?

CANADA

Reorganisation in Ottawa

A long-awaited bill on governmental organisation of scientific activities was finally presented to the Canadian Parliament shortly before Christmas. David Spurgeon reports from Ottawa.

THE bill proposes the reorganisation of the federal granting councils which provide funds for university research, and makes other changes concerning the Science Council of Canada and the Defense Research Board. At the same time Hugh Faulkner, Minister of State for Science and Technology, responded to concerns of the community about falling financial support, and said consideration is being given by the government to providing increases in 1977–78 budgets to cover inflationary increases in the cost of doing research.

The granting council changes were recommended several years ago by the Senate's special committee on science policy (the Lamontagne committee) and changes were promised by the government in February, 1974. According to Mr Faulkner, the bill is "the culmination of a lengthy series of studies, reports, meetings, and debates on the subject of science policy . . .".

The bill creates a Social Sciences and Humanities Research Council, leaving the Canada Council, which has supported both the arts and research in the social sciences and humanities, to deal entirely with the arts. Mr Faulkner said the reorganisation "reflects the growth in size and quality of the social sciences and humanities (in Canada) and the new perception of their importance to the attack on socio-economic problems, to national sovereignty, and to our cultural development". The bill also creates a Natural Sciences and Engineering Research Council, which will remove from the National Research Council of Canada the function of granting funds for university research in these areas. The NRC will henceforth deal with its own laboratories, industrial R&D support programmes, and a national programme of scientific and technical information. Mr Faulkner said the new council "recognises the need for a separate agency to support university research in these disciplines (natural sciences and engineering) and to foster improved dialogue between natural scientists and research engineers."

The Medical Research Council Act is amended under the new legislation to remove the restriction preventing its support of research in public health, but otherwise the council remains unchanged.

In order to advise and co-ordinate the work of all the granting bodies, Mr Faulkner will establish an Intercouncil Co-ordinating Committee, chaired by the Secretary of the Ministry of State for Science and Technology. The committee will include the presidents of the granting councils and will report to Mr Faulkner.

Although the criterion of excellence will continue to be the basis of university research support, additional funds will be provided to the councils for regional balance of scientific capability, national problems and support of inter-disciplinary research.

The Science Council's mandate is also restated in the legislation, to provide it with a greater national and public role. It has actually played such a role for years, but, according to the Minister, "the new mandate . . . will state clearly that the Council is to act as a source of public information and to provide advice on the profound effects developments in science and technology will continue to have on our way of life."

Amendments are also proposed to the NRC Act to give it flexibility to respond to national problems more readily when science and technology have a part to play. Provision has also been made to transfer the responsibility for Canadian Patents and Development Limited from the NRC to the Ministry of Industry, Trade and Commerce. This is supposed to make easier exploitation of inventions from government and university laboratories by industry. Thus the role of NRC is to change somewhat, with "less responsibility for the general development of science in Canada and more for using the demonstrated capability of its laboratories in tackling national problems."

● Towards the end of last year, the Lamontagne committee chairman, Senator Maurice Lamontagne, reported on progress to date in the committee's resurrected hearings, and the group resolved to go on and finish them in 1977. Senator Lamontagne said the current sessions have heard from 31 organisations and have accumulated about 1,300 pages of evidence. Six or seven more meetings are needed to conclude the hearings, which are concentrating on determining how many of the recommendations the committee made in 1972–73 have been followed by the government, and in what way. Mr Lamontagne noted that the current hearings have shown that many recommendations have been carried out, but that failure to follow some "is now producing what has been described to us as an acute crisis, endangering Canada's future capability in the whole field of scientific research, technological development and economic and social innovation." The two recommendations he noted had not been followed concerned the need to transfer older researchers to other jobs in the private and public sectors and the need for greater national expenditure on R&D. If the present situation continues, he warned, "Canada may lose a whole new generation of researchers". ☐

REACTION to the bill was not uncritical. Bill Kempling, Progressive Conservative MP for Halton-Wentworth, asked whether it was the first of several steps to eliminate the science ministry. He noted that MOSST can be cancelled at any time, without reference to Parliament, by an order in council. And he suggested that what the bill really does is increase the number of accounting, auditing and administrative personnel at a time when the government is talking about restraint in spending.

Mr Kempling noted that the intercouncil co-ordinating committee has no budgetary control and called it "another layer of bureaucracy." It would have been better to have set up an overall science foundation reporting to a full minister, with a number of divisions for different disciplines.

Cyril Symes, New Democratic MP from Sault Ste Marie, said it appears to him that whenever the Liberal government gets into trouble and has no real policy answers, "it carries out the old double shuffle: it reorganises parts of its bureaucracy with great fanfare to make it appear that it is doing something, whereas in actuality it is just shuffling the cards . . .". Mr Symes suspected that the bill was a sop to the lobbying of the science community, which had shown concern about funding cutbacks.

Paul Yewchuck, Progressive Conservative MP from Athabacsa, was concerned about the chairmanship of the intercouncil committee: "I can think of no good reason for the minister wanting one of his bureaucrats chairing the . . . committee, unless he thinks he can control the actions of that committee better if one of his underlings is in charge of it . . . I would rather see an active scientist taking this position."

creation of the monsoonal climate. This may in terms of land productivity be regarded as a marginal ecoclimate. The already marginal potential of most of the subcontinent became affected by the arrival of the Aryans with their browsing and grazing animals, their use of fire, and later of iron tools. The ecosystems in South Asia, with which the modern planners of use of arid and semi-arid lands have to cope, have been further reduced in potential in recent and modern times by the great increase in human and livestock populations, the latter due largely to politico-religious factors beyond the control of the land scientist.

In a region where the amount of arable land per person is estimated to have been 0.36 ha in 1950, 0.30 ha in 1965, 0.24 ha in 1975, and to be 0.19 ha in 1985, it is realistic to propose that any major developments in animal husbandry will be limited to marginal land which cannot produce cereal and legume grains for direct human consumption. In terms of the grazing and browsing resources of South Asia alone, the botanical composition of plant covers of the arid and semi-arid lands has deteriorated from a condition in which the superior perennial grasses provided reasonably good feed to livestock in the monsoon and subsequent months to less valuable grass covers of inferior perennials and annuals.

In the East, especially in Japan, Taiwan and China, there are large areas of sloping land, originally under a forest type in conditions between lowland monsoonal and upland temperate, which have been rendered marginal in terms of forest production and agriculture.

Matter of concern

To the conservationist, the ever-increasing area of land being rendered marginal is a matter of profound concern. Idealistic proposals for the total conservation of the environment are difficult to maintain with the population/land position in large parts of Asia. Marginal land *per se* generally represents a continuing and frequently increasing danger in terms of its own conservation, and of the conservation of soil and water on lands lower in the same catchment not yet rendered marginal. A realistic plan for conservation would embrace the preservation of natural biological ecosystems wherever this can be socially and economically justified. It would cover the reclamation of marginal lands either to their original condition or to a new balanced economic ecosystem in which sustained soil fertility and productivity can be maintained by the application of appropriate measures for conservation.

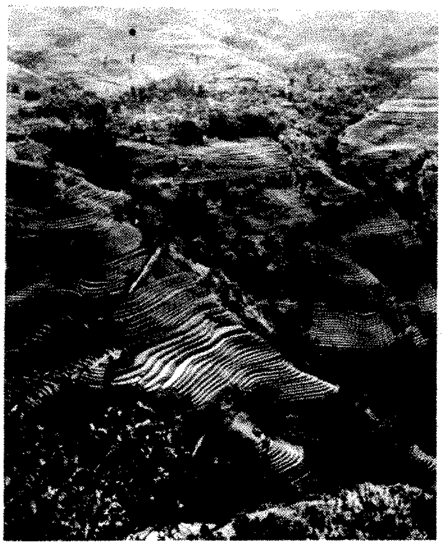
The reclamation of marginal land in either direction has so far been a matter of economics. The rural people responsible for making land marginal have had neither the knowledge nor the financial resources necessary for its correct treatment. Governments of Asian countries in which the return of marginal land from an unproductive to a productive state would be of great economic importance have not approached the problem with the energy it demands.

Again, this is largely because they apply the principles of conservation economics, of return upon capital investment, cost/benefit, input/output ratios and so on. But if most of the countries of Asia are at the age where it is the economics of survival which apply, a different set of criteria will be necessary if measures are to be applied on a sufficient scale.

The administrative structure of most Asian countries does not permit them to emulate the People's Republic of China, where in the winter of 1975–76 130 million rural workers were mobilised for what BBC monitors translated as farmland capital construction projects. These ranged from the reclamation of desert grasslands of Inner Mongolia and Sinkiang and the reforestation and levelling of land for irrigated cultivation in the eastern provinces, to the terracing of mountain slopes in the humid tropical island of Hainan.

Elsewhere some research is in progress. Increased productivity of marginal drylands is an important objective of the Indian government in association with the Canadian government, and at the International Centre for Research in the Semi-arid Tropics (ICRISAT), in Hyderabad. During the past six years the Central Soil Salinity Research Institute at Karnal, Haryana, has evolved economic and speedy methods for the reclamation of alkali soils of the Indo-Gangetic plains.

The essential components are land levelling, the application of gypsum or other additive, the application of fertilisers including micronutrients, the selection of suitable crops and crop varieties, and the adoption of suitable management practices. Semi-dwarf, high-yielding varieties of rice are ideally suited in the *kharif* (rainy) season, to be followed by wheat in the *rabi* (winter) season. Large-scale reclamation programmes based on this research are now being launched to reclaim lands which have been lying uncultivated.



A-CEI

Terraced fields in China

Delaying action

Foresters in the region are conducting a delaying action against rival claims on land for agriculture or for the grazing of livestock. The tropical rain forests present a major problem. Foresters still talk of the promotion after logging of natural regeneration on 100-year cycles, but this is rarely followed in practice. In Java, the relict vegetation on denuded land which might theoretically be reclaimed is used for fuel collection by thousands of people who have few alternative sources of income. Claims are made for the control of *Imperata cylindrica* by deep ploughing (Sumatra), replacement by sown fertilised pastures correctly managed to prevent regeneration (Sabah and the Philippines), or the use of systemic selective weed killers (Malaysia).

The marginal nature of the grazing and browsing resources of arid and semi-arid India are the concern of three research institutes, the Central Arid Zone Research Institute, the Indian Grassland and Fodder Research Institute and the Central Sheep and Wool Research Institute. These compare the relative merits of slow but cheap ecological progression and of reseeding with species of the local climax covers. A substation of the Japanese National Grassland Research Institute at Nishinasuno is developing methods for hill slope utilisation by the cheap method of hoof cultivation for the rearing of Holstein heifers for the dairy industry—a technique that may well be applicable in mainland China, Korea and Taiwan.

The reclamation and planning of future use of marginal land fall between a number of departments and institutes of central or state administrations. If these were to be regarded as a discrete aspect of land use the economic benefit would be great, particularly for the livestock and forest industries. That some type of joint action on a regional basis would be profitable, through ASEAN, the Asian Development Bank or the UN International Consultative Group for Agricultural Research, seems almost indisputable. ☐

Asia's marginal lands

Robert Orr Whyte examines the prospects for reclamation of an important natural resource

> There are no unproductive regions. There are only poor methods for cultivating land; there is no such thing as poor land. Provided only that people manifest in full measure their subjective capacities for action, it is possible to modify natural conditions.
>
> **Mao Tse-tung**

If the countries of monsoonal and equatorial Asia are to act upon the injunctions of United Nations and bilateral agencies and learn to feed themselves, they must utilise all their land and sea resources to the full. At present, there is insufficient knowledge of the real nature and magnitude of the land resources to show whether and to what extent this enormous target can be met. An assessment of land resources on modern lines is essential to confirm or adapt the existing land classes, and to define their relative areas and potential. The classes usually recognised are forests (protective or productive), grazing lands, uncultivable land other than forests, and cultivated land, whether used under systems of shifting cultivation, for plantation agriculture, or for dryland or irrigated crop production. However, one major land class which does not regularly appear in the statistics is marginal land.

Definition of the term depends upon the discipline of the speaker. Most of the uncultivated land, and much of the cultivated land that one sees in flying over the South Asian subcontinent or over the People's Republic of China, is marginal in nature. The agriculturist regards land as marginal when, because of terrain, soil or climatic conditions, the cultivation of arable crops becomes a risky enterprise, involving complete or partial crop failure in two or more years out of ten. The forester regards land as marginal when he cannot apply the appropriate silvicultural techniques to stands of natural vegetation, or when he cannot plant, maintain and harvest the produce from reforested blocks. Grazing land, particularly in arid and semi-arid regions, is marginal when it cannot supply feed of adequate quantity and quality to nomadic and migratory flocks and herds within a radius of daily walking distance, or when resources for stock watering are inadequate.

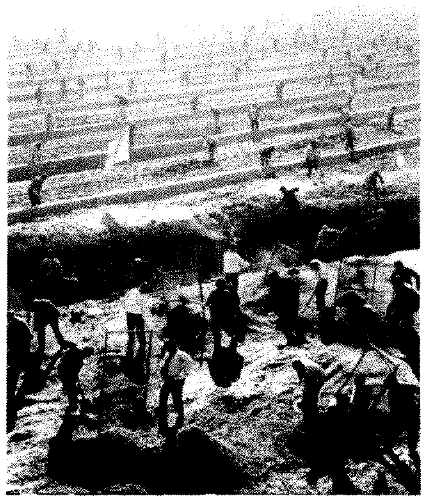
Land may be marginal in its natural state, or may have been rendered marginal due to the activities of man. The total area of marginal land of all types is not known. It is probably at least as large as the area under non-marginal cultivation at present. Only part of the marginal land of the region comes within the scope of the UN Environment Programme's Conference on Desertification, to be held in 1977.

It is debatable whether the greater potential for reclamation and increased production does not lie in the more densely populated countries of humid tropical Asia. The final criterion must be economic—whether the expense involved in bringing land back into economic use or in raising it from one plateau of production to the next above for the benefit of the local population can be justified under present conditions.

Attention to the problem of marginal lands must also be seen in correct perspective. It takes second place to the increase of productivity on existing cultivated land, which remains the great potential. The world average production of cereal grain per hectare is 1,898 kg. If the standards of Japan and other developed countries were to be achieved, the world output of cereals from existing cultivated land would be 3,286 million metric tons, or 859 kg per capita, or 95% of American consumption (see Lincoln Gordon and C. A. Pryor, *Science*, September 19, 1976). Yet we are still losing ground in this respect. In India, net availability per capita per day of cereals and pulses from domestic production was: 1956, 15.2 oz; 1974, 15.97 oz, and 1975, 14.65 oz.

Area increasing

The shifting cultivator has made the largest contribution to the man-made area of marginal land, and the area is still increasing rapidly year by year. Increased pressure of population on existing land resources and the progressive loss of traditionally exploited forest land to settled cultivators and timber extractors cause progressive reduction in the period of bush fallow. Regeneration of vegetation does not occur between successive periods of cultivation, and soil fertility deteriorates. What remains in humid tropical South-East Asia are pure stands of *Imperata cylindrica*, the 'green cogon deserts', much of which may still be reclaimed and can be regarded as marginal.



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Turning poor soil to farmland

An estimated 2.5 million hectares of potentially productive land are lying barren, because of high sodicity (alkali or sodic soils), in the west of the region and in the Indian States of Punjab, Haryana and Uttar Pradesh in particular. Inadequate surface drainage and consequent stagnation of water for prolonged periods, and a rise in the ground water table and accompanying physicochemical changes, appear to have resulted in the formation of these soils. These areas are currently grazing grounds for domestic livestock of low productivity or for abandoned bovines which have reverted to a semi-wild condition.

Probably at least comparable in area to the marginal lands created by shifting cultivation are the areas abandoned after deforestation. This happened in historical times in the Himalaya and their associated ranges and in China, and in recent and modern times in the Philippines, Indonesia and East and West Malaysia. Only limited areas have been reforested or transferred to plantation and arable agriculture in new economic ecosystems. These, however, rarely have the same conservation value as the biological ecosystems they have replaced.

The slaughter of the tropical rain forest in South-East Asia is eliminating the last remnants of a unique biological ecosystem, with its relatively unexplored genetic resources of plants and animals. And the areas from which marketable timber have been removed are left in such a condition after the operations of logging and extraction that further deterioration is inevitable, in terms of plant cover, soil erosion, desiccation of the environment and increased carbon dioxide content of the atmosphere—all factors not so far assessed accurately.

South Asia's marginality

The establishment of the drought-resistant plant and animal communities in the South Asian subcontinent, formerly tropical rain forest, followed the

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Science as an education

In this shortened version of a talk he gave recently on BBC Radio 3, Professor Sir Hermann Bondi, Chief Scientific Adviser to the Ministry of Defence, argues for a view of science as an education of the mind, rather than as professional training

My thesis is that science should be taught like the classics but has increasingly been taught like medicine. A medical education is a professional training with a practising doctor as end product. A classical education (and I would include more than just the classics here) has no such clearly defined end product, but is widely accepted as a good preparation for demanding tasks in administration and management. The reason is that students are taken through the thinking of some of the finest minds humanity has ever known, discuss and debate the difficult, often undecidable questions that these people discussed, and are trained to express themselves by word of mouth as well as by writing and to give a structure to their ideas, however complex or sophisticated the subject.

This is precisely the ability helpful in any senior task; in such a task one always deals with people and it is through argument and discussion, papers and analysis that one arrives at the kind of conclusion, at the kind of leadership, at the kind of consensus this necessary for achieving anything in administration and management. The only criticism must be that classics courses are very short indeed on numeracy, a skill which is of particular importance to administrators and managers who need to analyse statistics critically.

We have had a lot of trouble in recent years with young people not coming forward in the numbers expected to do science. This limited success of science in the all-important field of marketing an academic education is often ascribed, rightly, to the liking of young people to work with people rather than to deal in facts. This attitude reveals a misconception of what science is like, that to me is simply staggering. Science doesn't deal with facts; indeed, fact is an emotion-loaded word for which there is little place in scientific debate. Science is above all a cooperative enterprise. It is a social activity which is perhaps more successful than any other in enabling people of different religion, politics, race, culture and ideology to work together, and it is thus a particularly human subject.

Science reveals its humanity most in those research issues that are the subject of current debate, discussion and seminar. In these fields there is no certainty, only arguments. These arguments can indeed be supported in an exceptionally important way by experiment, but can also be disproved by a sufficiently careful and ingenious experiment or observation. Science is thus a field in which imagination is of the utmost importance: not, it must be emphasised, untrammelled imagination, but imagination carefully controlled by what can and cannot be tested experimentally.

> 'It ill-becomes scientists to deplore the fact that so few of them are in parliament, the upper reaches of the Civil Service, the banks, the highest positions in industry. For have science courses been aimed to produce such people?'

When we look at science, not so much as a profession but as an intellectual adventure, it is clear that it has had some of the finest minds that mankind ever produced, grappling with problems a little harder to define than some of the philosophical problems of the ancients, but also problems where, through the test of experiment and observation, human imagination has been brought to its highest peak. This aspect of science does not come across anything like well enough to attract young students.

If it is educational for performers of senior and responsible tasks to be taken through the thoughts of a Plato, is it not just as good, perhaps even better, for them to be taken through the thoughts of a Newton or an Einstein? I say better because of the additional demands of numeracy. I claim that if we conceive of science as a group of subjects offering an education in thinking, communication and particularly the evolution of human ideas generated by an unequalled creativity, originality and imagination, then the education that can be provided is unsurpassed.

Hitherto the voracious appetite of government and industry for scientists during the 1950s and much of the 1960s propelled the universities into producing the maximum number of professional 'bench' scientists they could, which was thought, to some extent at least, to involve stuffing as much scientific knowledge into them as was possible. I would not wish it to be thought that I view those efforts—in sime of which I partook—as bad or disadvantageous; only that the emphasis was confined, incorrectly, to the aim of producing professional scientists.

If one had viewed science as an education of the mind rather than as professional training, naturally students emerging with a first degree would have known less science. But they would have known more about the kingdom of scientific ideas, about how to argue, about the evolution of human thinking and about how scientific debate and the interaction of experiment and theory stimulate creativity.

Further, such an orientation of science courses might also produce better scientists, first because such teaching would attract many of the ablest young people now repelled by the supposedly inhuman face of science, and second because in science itself an ability to argue, debate, and understand the other person's ideas, however imperfectly expressed, is at least as important as sheer scientific knowledge.

It ill-becomes scientists to deplore the fact that so few of them are in parliament, the upper reaches of the Civil Service, the banks, the highest positions in industry. For have science courses been aimed to produce such people? There have been a number of efforts in the past ten or fifteen years to improve the balance. They have had most creditable success in establishing novel courses, but these only affect a very small proportion of the science students and therefore do little to alter the prevailing misconception of what science is.

I do not want it to be thought that science as an education is intellectually less demanding than an education in science. Quite the contrary. And I certainly would not wish to propagate a course that was not really demanding, because that would fail in its purpose: attracting the ablest young people to what is the most exciting area of human enterprise. ☐

nature

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Ford leaves a healthy legacy

GERALD FORD will no doubt be recorded in the history book as a President who, in his brief term of office, performed an extraordinary act of national healing, pouring a good deal of soothing balm on the political wounds inflicted by his predecessor. He did nothing too flamboyant, pursued few major new initiatives, and left the overall structure and operations of the federal government virtually intact. That pretty well sums up his record in science policy as well.

When he entered the White House two-and-a-half years ago, relations between the federal government and that slice of the population known as the scientific community were at an all-time low. When he handed over the reins to President Carter last week, the management and funding of federal science had been placed on a firmer footing, and the scientific community was, if not satisfied, at least mollified.

Mr Ford's most conspicuous act of healing in science affairs was the reinstatement of a science policy office in the White House, a move which has won much praise even though the new office came into being a scant five months before Mr Ford left Washington. Few of Mr Nixon's acts caused as much bitterness among scientists as his decision in 1973 to abolish the Office of Science and Technology and the President's Science Advisory Committee, bodies which had been providing scientific advice to the White House for more than a decade. Mr Ford's decision to reinstate a science advisory apparatus in the White House, though it took most of his Presidency to accomplish, has laid the basis for more rational scientific planning.

As far as budgets for research and development are concerned, Mr Ford has again placed matters on a relatively sound basis. When he came to power, he inherited a dismal state of affairs. During the previous few years, total federal support for research and development had declined as inflation more than gobbled up the small dollar increases proposed by Mr Nixon. Basic research was especially hard hit, with federal support declining in real terms by more than 20% between 1969 and 1973. And superimposed on the slow downward drift was a debilitating series of abrupt oscillations in funds for some programmes as Mr Nixon attempted to put a hold on public expenditure and to shift funds into priority areas at the expense of less politically favoured programmes. During Mr Ford's brief Presidency, there has been some real growth in funds, and basic research has been given a welcome boost.

The growth was particularly surprising because the three budgets which Mr Ford submitted to Congress all reflected his overriding concern to curb the overall rate of growth in the federal budget; science was given a financial boost while other programmes, particularly those in health and welfare, were held back. And Mr Ford's last budget, delivered to Congress just three days before his departure, continued the trend and even added some long overdue support for new research programmes in agriculture and the earth sciences.

But that does not mean Mr Ford has solved all the problems in federal support of research and development, and that he is handing President Carter a trouble-free scientific enterprise. Far from it.

Energy policy is in a mess, and oil imports are actually higher now than they were before the 1973 embargo. The overall strategy which has marked both the Nixon and Ford energy policies was to throw massive amounts of money into energy research and development, particularly nuclear technologies, in the hope of increasing energy supplies. Little attention has been paid to curbing demand, either through regulation or research on energy-saving technologies, and President Carter has rightly criticised the outgoing Administration for its failure to establish a coherent energy policy. A particular failure has been the Ford Administration's attempts to curb nuclear proliferation, where policies have been thrown together in response to crises rather than developed by careful planning.

In the area of biomedical research, Mr Ford inherited a particularly dismal situation, and though he has made some progress, he is leaving his successor a number of problems. Two chief factors have led to disarray in biomedical research planning. The first was the passage of the National Cancer Act, which has caused funding for cancer to balloon while support for many other areas of research has languished. Although Mr Ford has tried to redress the balance in his budgets, he did not acknowledge, let alone implement, some modest recommendations for reform published last year by a Presidential commission.

The second principal area of irritation has been a continuing struggle between Republican Administrations and the Democratic Congress over the size of the budget for the Department of Health, Education and Welfare. Presidential vetoes and refusals to compromise on both sides have greatly delayed the passage of appropriations bills and caused federal funds to flow into the National Institutes of Health in a series of surges and trickles. Mr Ford's budgets, including his final proposals, have perpetuated the trend.

Nevertheless, on balance, Mr Ford is leaving his successor a surprisingly healthy scientific legacy, and few major changes in the federal science policy should be anticipated from the new Administration, at least in the next year or so. □

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Publications of the Finnish Geodetic Institute. No. 80: Precise Computation of the Precession. By R. A. Hirvonen. Pp. 25. Reports of the Finnish Geodetic Institute. 76:4: An Automatically Operated Weather Station. By J. Kakkuri and Kari Kalliomäki. Pp. 19. 76:5: Stellar Triangulation Points—Niinisalo, Tuorla and Naulakallio. By R. Konttinen. Pp. 13. (Helsinki: Finnish Geodetic Institute, 1976.) [2911

Advances in Research and Technology of Seeds. Part 2. Edited by J. R. Thomson. Pp. 112. (Wageningen: Centre for Agricultural Publishing and Documentation 1976.) Dfl.25. [2911

Plague Manual. By M. Bahmanyar and D. C. Cavanaugh. Pp. 76. (Geneva: World Health Organization; London: HMSO, 1976.) Sw.fr.20. [2911

CERN—European Organization for Nuclear Research. CERN 76–18: Physics with Very High Energy e^+e^- Colliding Beams. By L. Camilleri, *et al*. Pp. 352. (Geneva: CERN, 1976. 2911

announcements

Appointments

Professor C. C. Booth has been appointed by the Medical Research Council to succeed Sir Graham Bull as Director of the Clinical Research Centre, Northwick Park, Harrow.

Awards

The **Paul Martini Award** has been increased to 20.000 DM. The award is for development of scientific methods for evaluation in the clinical, pharmacological and therapeutic fields. Papers should be sent with six copies to the Medizinisch Pharmazeutisch Studiengesellschaft e.V., Humboldtstrasse 94, D 6000 Frankfurt. The competition deadline will be April 30, 1977.

Meetings

March 23–25, **Nuclear Physics,** Surrey (Institute of Physics, 47 Belgrave Square, London SW1).

March 23–25, **The Challenge of Change,** York (Institute of Information Scientists, S. Nichol, 'Rivendell', Southfield Road, Driffield, North Humberside).

March 26–April 5, **Genetics, Evolution and Ecology of Marine Organisms,** Venice (J. A. Beardmore, University College of Swansea; P. Battaglia, University of Padua).

March 28–April 1, **Chemistry in Our Lives,** London (The Chemical Society, Burlington House, London W1).

April 4–7, **Displays for Man-Machine Systems,** Lancaster (Institution of Electrical Engineers, Savoy Place, London WC2).

April 15–16, **Stadler Genetics Symposium,** Columbia, Missouri (117, Curtis Hall, University of Missouri, Columbia Mo 65201).

May 3, **Crop Protection,** Gent, Belgium (Ir. W. Dejonkheere, Agricultural Sciences, University of Gent, Belgium).

May 25–27, **The Testis,** Toronto (I. B. Fritz, University of Toronto, 112 College St, Toronto, Ontario M5G IL6, Canada).

May 31–June 2, **Pharmaceutical Technology,** Paris (Association de Pharmacie Galenique Industrielle, Rue J. B. Clement 92290 Chatenay-Malebry, France).

June 1–3, **Frequency Control,** Atlantic City, New Jersey (J. R. Vig, US Army Electronics Command, Fort Monmouth, New Jersey 07703).

July 5, **Solar Energy Conversion,** London (P. T. Landsberg, The University, Southampton).

July 17–20, **International Metallographic Society,** Houston, Texas (IMSI, Evanston, Illinois 60204).

July 25–28, **Myxobacteria,** Norwich (J. H. Parish, Biochemistry, University of Leeds, UK).

August 15–17, **Endocrine Functions of the Testis,** Austin, Texas (Society for the Study of Reproduction, 113 North Neil St, Champaign, Illinois 61820).

August 21–September 3, **Genome Organisation and Function,** Spetsai, Summer School (H. G. Zachau, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie, Goethestrape 33, D-8000 München 2).

August 23–26, **Chemical Thermodynamics,** Ronneby, Sweden (IUPAC Conference, S. Sunner, Chemical Centre, Lund University, PO Box 740, S-22007, Lund, Sweden).

August 24–26, **High Polymers, Ottawa** (J. Roovers, 19th Canadian High Polymer Forum NRCC, Division of Chemistry, Ottawa, Ontario KIA OR9, Canada).

September 1–2, **Cancer Immunotherapy and Prophylaxis,** Bucharest (Oncological Institute, P.O. Box 5916, Bucharast 12, Romania).

September 5–9, **Precise Electrical Measurement,** London (Institution of Electrical Engineers, Savoy Place, London WC2).

October 2–8, **Physical and Chemical Information Transfer in Regulation of Reproduction and Ageing,** Varna, Bulgaria (J. G. Vassileva-Poppova, Biophysics, Bulgarian Academy of Science, 1113 Sophia, Bulgaria).

October 17–20, **European Society for Radiation Biology,** Liège, Belgium (R. Goutier, 32 Boulevard de la Constitution, B-4020 Liège, Belgium).

October 31–November 4, **Radioimmunassay,** Berlin (E. H. Belcher, Medical Applications Section, IAEA, Kartner Ring 11, PO Box 590, A 1011, Vienna, Austria).

April 4–7, 1978, **Communications Equipment and Systems,** Birmingham (Institution of Electrical Engineers, Savoy Place, London WC2).

Person to Person

Surrey–Minneapolis, St Paul. 4-bedroomed house in Frensham near Farnham, Surrey, offered in exchange for similar sized house in Minneapolis, St Paul, from March 1977 for one year. Frensham is a village in delightful countryside within commuting distance of London by train. The Universities of Surrey and Reading are within 30-min drive. Contact Dr J. N. Gibbs, Dragon Lodge, Millbridge, Frensham, Surrey.

Wanted: copy of *The History of the Study of Landforms,* vol. 1, *Geomorphology Before Davis,* by R. J. Chorley, A. J. Dunn & R. P. Beckinsale (Methuen, London, 1964). Please state condition and price to Dr P. J. Smith, Department of Earth Sciences, Open University, Walton, Milton Keynes, UK.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The **section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.**

Reports and Publications

UK & Ireland–November

National Radiological Protection Board. NRPB R42: Radiological Protection Tests for Products which can Lead to Exposure of the Public to Ionising Radiation. By Marion D. Hill, A. D. Wrixon and B. T. Wilkins. Pp. 16. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1976.) [111

Proceedings of the Royal Irish Academy. Vol. 76, Section B. No. 19: Bird Communities of some Killarney Woodlands. By L. A. Batten. Pp. 285–314. 87p. No. 20: Variation in Invertebrate Community Structure in Three Freshwater Habitats in Ireland, as Described by an Index of Species Diversity. By E. Fahy. Pp. 315–322. 42p. No. 21: A Woodland Population of Small Rodents (*Apodemus sylvaticus* (L) and *Clethrionomys glareolus* Schreber) at Adare, Co. Limerick. By J. S. Fairley and J. M. Jones. Pp. 323–336. 63p. No. 22: Explosion Breccias and Related Features in the Leinster Caledonian Massif, South-East Ireland. By J. C. Brindley, L. N. Gupta and P. S. Kennan. Pp. 337–348 + plate 12. 60p. (Dublin: Royal Irish Academy, 1976.) [211

University College of Wales, Aberystwyth. Report of the Welsh Plant Breeding Station for 1975. Pp. 145. (Plas Gogerddan, Near Aberystwyth: Welsh Plant Breeding Station, 1976.) £1. [311

Environmental Conservation in Action: Oil and Gas. (Shell Briefing Service.) Pp. 8. (London: Shell International Petroleum, Co., Ltd., 1976.) [311

A Correlation of Dinantian Rocks in the British Isles. By T. N. George, G. A. L. Johnson, M. Mitchell, J. R. Prentice, W. H. C. Ramsbotton, G. D. Sevastopulo and R. B. Wilson. Pp. 87. (Special Report No. 7). (London: Geological Society of London, 1976.) £3.50; $7.50. [511

Potato Marketing Board. Annual Report and Accounts 1976. Pp. 36. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1976.) [811

The Family Planning Association. 44th Report and Accounts, 1975–1976. Pp. 29. (London: Family Planning Association, 27/35 Mortimer Street, W1, 1976.) [811

The British Library. Science Reference Library. Periodicals on Ornithology held by the SRL. Pp. 14. (London: Science Reference Library, 1976.) [811

Edwards Freeze-Drying Handbook. By Terence W. G. Rowe and John W. Snowman. Pp. 113. (Crawley: Edwards High Vacuum, 1976.) [811

An Illustrated Key to Freshwater and Soil Amoebae with notes on Cultivation and Ecology. By F. C. Page. Pp. 155. (Scientific Publication No. 34.) (Ambleside, Cumbria: Freshwater Biological Association, 1976.) £2.50. [1011

Bare outlines of medicine

Molecular and Cellular Mechanisms in Disease. Vol. 1: Pp. xv+1–604+xxvi. Vol. 2: Pp. ix+605–1116+xxvi. By Julian L. Van Lancker. (Springer: Berlin and New York, 1976.) DM362, $148.50.

After some random reading here and there in the two volumes which make up this enormous monograph, I began to experience an extraordinary sensation of *déjà vu*. It was easy to trace this sensation to my memory of an earlier, equally enormous monograph on cellular pathology. The relationship between the two may be likened to that between the very 'with it' grandson of a highly respectable grandfather, with the grandson remaining very much a pillar of the family firm. Every generation of pathologists seems to produce one who cannot resist the temptation of trying to put down in one tome all knowledge that could possibly pertain to the subject, presumably in the hope of making it more comprehensible. This is in the tradition of Virchow and Cohnheim. In their day one man could still know enough facts in all the very different facets of pathology with sufficient accuracy to think about their interrelationship fruitfully. That has long since become impossible.

This book, therefore, demonstrates the case against a multi-topic textbook written by a single person. An almost unbelievable erudition is incapable of preventing errors of fact and distortions in interpretation, usually as a result of oversimplifications, which are serious enough to vitiate any supposed advantage arising from the unity of one man's outlook. In the arts a wide-ranging survey by one percipient mind may be singularly stimulating; thus, the *Outline of History* by H. G. Wells is for many an unforgettable intellectual experience. That this is still possible in the natural sciences is shown by such books as Jacques Monod's *Chance and Necessity* or Richard Dawkin's *The Selfish Gene*. But these are books of ideas, not compendia of information; and therein lies the difference between monographic success and failure.

In the preface the author says: "The book cannot be comprehensive because too little is known about the mechanisms of disease, and no-one could hope to master all available knowledge." So the puzzle is why the attempt was made to do just that. The comprehensiveness of the attempt is shown by the chapter headings: cellular sources of energy; determination of cellular specificity; inborn errors of metabolism; malnutrition; calcium and phosphorus metabolism; iron and bile pigment metabolism; blood coagulation; hormones; alterations of body fluids and electrolyte metabolism; cellular death and degeneration; blood lipids in arteriosclerosis and hyperlipidemia; radiation; inflammation; immunopathology; regeneration, hypertrophy, and wound healing; cancer.

The only way in which someone with much else to do can assess such a book is by reading carefully sections in which his own knowledge is as accurate as possible. Thus, the chapters on blood coagulation, hormones, blood lipids in arteriosclerosis and hyperlipidemia, and inflammation contain much useful, standard information, but any serious student would have to be provided with accurate, up-to-date reviews to correct impressions produced by the book. For example, many of the statements on page 410 are erroneous in fact or interpretation; on page 767 consideration of increased capillary permeability is entirely morphological and non-quantitative, and ignores all physiological and quantitative descriptions; on page 771 there are still discussions of "leukotaxine" and "exudine" which might have been assumed to have been buried long ago; and on page 775 and beyond, the account of the prostaglandins is necessarily already out of date. Indeed, such a book is bound to be out of date by the time it appeared. The references seem to be mainly to the 1960s although some reach as close to the present as 1973 and 1974; and there are references to truly historical papers. There is no harm in that—on the contrary, students nowadays know much too little of the history of their subjects—as long as it does not result in underemphasis of modern developments.

According to the preface, the book is addressed to students of disease whether they are clinicians or researchers. "Hopefully, it will contribute to making medicine more of a science, guide the investigator in unexplored territory, and help the practitioner respond to the request of Macbeth, '. . . find her disease/And purge it to a sound pristinge health'." It seems sad to have to conclude that this book will not achieve what the author hopes of it.

G. V. R. Born

Professor Born is Sheild Professor of Pharmacology at the University of Cambridge, UK.

Contemporary immunology

Manual of Clinical Immunology. Edited by N. R. Rose and H. Friedman. Pp. xxv+932. (American Society for Microbiology: Washington, August 1976.) Hardback $20; paperback $16.

The burgeoning of immunology in the past twenty years has caused alarm to granting bodies and to its increasingly confused protagonists. The grass roots of this monster growth are to be found in methods of vaccination, blood group typing and the various serodiagnostic and classificatory methods of microbiology. Since the last war the successive discoveries of cell-mediated immunity, immunological tolerance, the recirculation of the lymphocyte, a function for the thymus and the fine structure of immunoglobulin molecules have effectively nourished the somewhat desiccated root disciplines and led to the rather top-heavy state of contemporary immunology.

The present volume is a blend of applications of the new immunology with up-to-date versions of some of the older approaches. It is sad that relatively little space is given to epidemiological studies, to vaccination technology and strategy, to the special problems of immunological staging of cancer patients, to the quantitation of lymphocytes by reference to their origins and to parasitic diseases. It may be, however, that some of these topics are already well covered in other large books. The emphasis of the book is avowedly practical, and as a manual it is remarkably useful even if not comprehensive.

The book is split into eleven major sections covering fields such as "Transplantation immunology", "Immunoassays", "Autoimmune diseases" and "Humoral immunity". There is a welcome section on the organisation and development of clinical immunological facilities. Each section consists of a number of papers by specialists in the field. It is claimed that enough information is given about techniques for an experienced technologist to carry out a procedure which was new to him (or her) without further search of the literature. As far as I can judge the chapters are clearly and concisely written with snags and deficiencies exposed to view.

It would be nice to see a book like this with the pages detachable on metal rings and with a visible index if it is really intended as a laboratory manual. The book is, however, good value.

A. J. S. Davies

A. J. S. Davies is Professor of Immunobiology in the University of London, working at the Chester Beatty Research Institute, London, UK.

Drosophila genetics

The Genetics and Biology of Drosophila. Volumes 1a, b, c. Edited by M. Ashburner and E. Novitski. Pp. xv+486, xv+487–954, xii+955–1,427. (Academic: New York, San Francisco and London, 1976.) £16.80 per volume.

THE present three volumes represent a substantial part of an ambitious treatise dealing with the genetics, biology, development, evolution and ecology of *Drosophila* in the form of a series of articles contributed by specialists in the various subdivisions of this extensive field. They deal with the more strictly genetic aspects and display the great variety of analytical techniques which can be applied, especially in *D. melanogaster*. Ever since the early days of *Drosophila* genetics, presided over by the now legendary associates of T. H. Morgan, there has been a progressive development of ingenious ways of exploiting structurally altered chromosomes to investigate the properties of genes and chromosomes, often combined with supporting evidence from the ordered banding of the polytene chromosomes of the salivary gland. This methodology is dealt with in the articles according to their relevance to particular topics, but it is a pity there is no classified account of the principles of analysis and how they may be applied to different kinds of problem. This would have been valuable for newcomers to *Drosophila* and would have reduced the sense of discontinuity created by the rather arbitrary ordering of topics. Is it too much to hope that such an article could be included in a later volume of this series? Might the editors be persuaded to round off their labours with what could well be a *tour de force*?

In the total of some 1,400 pages the articles deal with such topics as chromosome aberration, conditionally expressed mutations, mosaic systems, gene conversion, meiotic mutants, distributive pairing, compound and ring chromosomes, altered segregations including segregation distortion, genetics of the Y chromosome, analysis of simple and complex loci, analysis of the proximal region of the X chromosome and of the fourth chromosome, position effect variation, mitotic recombination, the bobbed locus, radiation genetics, chemical mutagenesis, polytene chromosomes, together with reviews of *D. virilis* and *D. hydei*.

Merely to list these topics, which are generally covered in depth, is sufficient to indicate the wealth of information and conceptual diversity packed into these volumes. Naturally, almost all the theoretical framework has been strictly genetic in origin; ultimately it will have to be reconciled with molecular evidence and interpretation, and a promising start has been made in that direction. Studies of the kinetics of extracted DNA, its appearance under the electronmicroscope, DNA–DNA and DNA–RNA hybridisation, the sequencing of satellites, *in situ* hybridisation to the polytene chromosomes and the characterisation of transfer RNA have already made a notable contribution. The elegant analysis of the bobbed locus described here is an excellent example of how the classic *Drosophila* methodology can be integrated with the analytical methods of nucleic acid biochemistry. Current developments in DNA sequencing will surely guarantee that the *Drosophila* genome will continue to be better known than that of any other eukaryote.

This interest in the use of the analytical tools provided by *Drosophila* is not confined to molecular biologists. In the past decade students of embryonic development and differentiation, cell lineage in organ formation, behaviour, and the biochemistry of gene action have discovered in *Drosophila* strategically significant situations worth investigating.

One of the pleasing features of this work is that the historical aspect is not neglected. There is an introductory chapter by C. P. Oliver, one of the *Drosophila* veterans, which outlines the chronology of the major discoveries. In many of the articles there is comment on the origin of new interpretations or techniques and the reader is often thus reminded of the friendly exchange of stocks and ideas which has been, and remains, a traditional attitude among *Drosophila* workers.

It is obvious from the list of 44 contributors to the first three volumes of this series, that the centre of gravity of *Drosophila* research lies within the confines of North America, not overlooking some important European laboratories. It is a pity there are so few British laboratories in which the *Drosophila* lore flourishes. Perhaps these historic volumes will encourage some of our younger biologists to invade the field, provided they have a chance of reading them, none too certain in these days of dwindling library grants in view of the regrettably formidable price per volume.

It must have required immense effort, patience and tact on the part of the editors to marshall so many authors and ensure that the contributions were sufficiently similar in style and standard. They are to be congratulated on their altruistic devotion which will be gratefully acknowledged for many years to come. **Forbes W. Robertson**

Forbes Robertson is Professor of Genetics at the University of Aberdeen, UK.

Marine pollution

Effects of Pollutants on Aquatic Organisms. (Society for Experimental Biology, Seminar Series 2). Edited by A. P. N. Lockwood. Pp. x+193. (Cambridge University: Cambridge, London and New York, September 1976). Hardcover £8.50; paperback £3.80.

THE overall trend towards physiological effects and turnover is apparent from most of the papers collected in this volume. Heavy metal storage, excretion and tolerance is covered comprehensively by Bryan with considerable experimental data on *Nereis*. The effects of cadmium on fish biochemistry is described by Larsson *et al.* Cautious comparisons are made with published work in the mammalian field.

Fish physiology is taken up further by Lloyd and Swift. Gas exchange and water balance are discussed with some emphasis on the care required in explaining observed abnormalities in fish kept in captivity. A quantitative approach towards histological studies in gills is presented by Hughes along with interesting information on fish respiratory and circulatory mechanisms. Support is expressed for the tentative hypothesis of Lloyd, that increased ventilation rate by the gills increases the toxicity of poisons.

Organochlorine compounds are dealt with in a detailed review by Addison, in which the author makes several helpful suggestions for future work. The effects of hydrocarbons are covered in two papers. Corner *et al.* comprehensively cover zooplankton and fish. Vandermeulen and Ahern appropriately deal with the primary producers, being concerned with algal physiology. Both papers include experimental data on the effects of naphthalene on these organisms. Zooplankton and phytoplankton are discussed again by Reeve *et al.* who describe the first year's results obtained in the ambitious 'Controlled Environment Pollution Experiment'. Interesting comparisons with laboratory-acquired data are presented with particular reference to copper.

The wide coverage of this book, its readability and excellent index, will make it valuable for project hunters and established researchers alike.

G. C. Carney

Dr Carney is a Lecturer in the Department of Science at Bristol Polytechnic, UK.

WILEY
INTERSCIENCE

Water, Wastes and Health in Hot Climates

edited by **R. Feachem,** *Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine;* **M. McGarry,** *International Development Research Center, Ottawa,* and **D. Mara,** *Department of Civil Engineering, University of Dundee.*

This book is a multi-disciplinary approach to the problems associated with water and wastes in hot climates, particularly those of the tropical developing countries. Solutions for these types of problems in hot climates have generally been modelled on practices adopted in industrialized countries with temperate climates which are invariably inappropriate. This book provides a basis for the interdisciplinary activities required to make the best use of the resources available in sanitary engineering in the Third World.

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by **C. F. Baes, Jr.,** and **R. E. Mesmer,** *both of Oak Ridge National Laboratory.*

A critical review of hydrolysis species and their stability constants in aqueous solution. Critically evaluates the various information on the identities and stabilities of species in solution as well as the solid hydroxides and oxides produced there. Summarizes selected values of stability quotients and surveys trends.

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Chemistry of Marine Sediments

edited by **T. F. Yen,** *Faculty of Engineering Sciences and Chemical Engineering, University of Southern California.*

A new volume on a topic of growing importance written by authorities in their fields and covering a wide variety of problems, methods and waters.

0250 40103 7 approx. 300 pages In Press
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Published by Ann Arbor Sciences Inc., and distributed by John Wiley & Sons Ltd.

Methods of Biochemical Analysis Vol. 23

edited by **D. Glick,** *Stanford Research Institute.*

Continues the series objective of presenting subjects of current importance in the field with experimental details sufficient to enable the laboratory worker to carry out the analysis under discussion.

0471 01413 3 446 pages December 1976 £19.20/$31.00

Paleobiogeography

edited by **C. A. Ross,** *Western Washington State College.*

edited by **C. A. Ross,** *Western Washington State College.*

Explores the development of old and new ideas that form the bases for present concepts about the world's biogeographic past. Outlines the perspective of paleobiogeography and examines recognition of distinctive parts of a biota and their dispersal, phyletic lineages and their origins, and the permanency of oceans and continents.
(Benchmark Papers in Geology).

0470 15135 8 446 pages September 1976 £20.15/$34.15

Published by Dowden, Hutchinson and Ross Inc., and distributed by John Wiley & Sons Ltd.

Fundamentals of Soil Behavior

by **J. K. Mitchell,** *University of California, Berkeley.*

Combines principles of geology, mineralogy, soil science, chemistry, and physics to provide a basis for understanding the engineering properties of soils, the factors controlling their magnitude, and the influences of environment and time.

0471 61168 9 440 pages April 1976 £17.60/$30.30

Interpreting the Environment

edited by **G. W. Sharpe,** *University of Washington.*

A collection of articles by specialists on parks, national forests, and other such areas. Essays examine the professional development of the interpretive programme, the objectives of interpretation, the methods and techniques used, and the personnel implementing the programme.

0471 77896 6 582 pages February 1976 £10.80/$18.35

Environmental Toxicity of Aquatic Radionuclides: Models and Mechanisms

edited by **M. W. Miller,** *Department of Radiation Biology and Biophysics,* and **J. N. Stannard,** *School of Medicine and Dentistry, both of the University of Rochester.*

This is the proceedings of a conference of international experts concerned with the problem and evaluation of aquatic radionuclide toxicity. Topics considered include the biogeochemistry of transuranic radionuclides, the evaluation of actual measurements and their applicability to model making for radionuclide release and distribution, and the general ecological factors of radionuclide toxicity.

0250 40132 0 350 pages December 1976 £18.85/$29.15

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Randon Measures

Olav Kallenberg

1977, 104pp., £6.00/$10.00 0.12.394950.5

Randon measure theory is a new and rapidly developing branch of probability, being of increasing importance both in applications and as a tool in other theoretical fields. Although, historically, most results of the theory were first discovered in the point process case, the more general framework of random measures seems to provide the natural scope of a theory.
The present book gives a concise and systematic account of some basic ideas of the subject, and emphasises questions of uniqueness and convergence in distribution, as well as the notions of infinite divisibility, null-arrays, symmetric distributions, Palm distribution, and mixed Poisson and sample processes.

European Monographs in Social Psychology Number 11
Series editor: H. Tajfel

Linguistic Style and Persuasion

Rolf Sandell

February/March 1977, xiv + 330pp., £10.50/$23.00 0.12.618150.0

In a world where mass communication plays an important part in our attitudes towards people, ideas, events and objects, the wording of a message may well be the most significant factor in persuading us to adopt a particular point of view. How susceptible are we to linguistic style? How profoundly can the impact of a message be enhanced by the manner in which it is written? This stimulating study approaches these questions from an entirely fresh viewpoint. Instead of using a general psychology of language, the author introduces "psychostylistics", the differential psychology of language which outlines a new pathway to the understanding of language as a manipulatory tool.

The Plasma Membrane

Models for Structure and Function

B. D. Gomperts

February/March 1977, xx + 256pp., £11.40/$22.25 0.12.289450.2

In the fast expanding field of membrane science, there now exists a need for specialised monographs at an introductory level devoted to selected aspects. This book brings together a wide experience of the analytical and synthetic approaches to membrane structure with the aim of showing how these may be applied to more general problems of membrane function and mammalian cell biology, and lays great stress on the development and use of model structures in the investigation of membrane structure and function. This monograph is probably the first extensive discussion of this important field to appear at this level; it illustrates how a firm understanding of model systems can be used to unravel complex problems in cell biology, and includes a discussion of work emanating from the author's own laboratory on the mechanism of cellular activation in a secretory system.

The Analysis of Organic Materials An International Series of Monographs Number 9

Aldehydes — Photometric Analysis Volume 4

Eugene Sawicki and Carole R. Sawicki

January/February 1977, x + 288pp., £12.00/$26.25 0.12.620504.3

Here is the fourth in this comprehensive series on the photometric analysis of aldehydes. It discusses the photometric analysis of precursors through their derived aldehydes and also the formation of aldehydes through a variety of reactions. In the future, the latter could be of increased importance in the development of methods of analysis utilizing photometry, gas chromatography, high performance liquid chromatography and mass spectrometry.

Advances in Aquatic Microbiology Volume 1

edited by M. R. Droop and H. W. Jannasch

March/April 1977, approx. 350pp., £14.50/$31.75 0.12.003002.0

Contents

S. I. Kuznetsov: Trends in the development of ecological microbiology. *C. M. Brown and B. Johnson:* Inorganic nitrogen assimilation in aquatic microorganisms. *C. R. Curds and M. J. Bazin:* Protozoan predation in batch and continuous culture. *W. D. P. Stewart and M. J. Daft:* Microbial pathogens in cynaophycean blooms. *Vera G. Collins:* Methods in sediment microbiology. *G. T. Bonde:* Bacterial indication of water pollution. Subject index.

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Circle No. 14 on Reader Enquiry Form.

Autonomic nervous system

Structure of the Autonomic Nervous System. By G. Gabella. Pp. viii+214. (Chapman and Hall: London; Halsted: New York, June 1976.) £9.80.

DR GABELLA has produced an informative account of the structure of the autonomic nervous system. It is given in small compass (160 pages), and the author has also restricted his historical introduction to just over a page. The book then examines the structural anatomy of sympathetic ganglia and peripheral sympathetic nerves, including their development and regeneration. Parasympathetic structures, including the ciliary ganglia and the vagus nerve, are then described. The last chapter is devoted to the innervation of organs, including the eye, salivary glands, skin, adipose tissue, gut, the heart, blood vessels, respiratory tract and the genital tracts. The monograph is well supported by 800 references which are helpfully arranged at the end of the book in alphabetical order .The references quoted are recent, and many have been published in the 1970s up to 1975. The information is based on comparative anatomy and there is frequent mention of the species on which the observations have been made.

Thus the book provides well documented information on the structures described, and will be valuable as a source of reference to anyone who is dealing with the structural anatomy of the autonomic nervous system in animals as well as man. The book has, however, some limitations. On the production side it seems unfortunate that the error in binding—the plates have been incorrectly placed—should not have been corrected, so that the publishers have only noted the error with an erratum slip. It is nevertheless a well produced and attractive volume. There are also some deficiencies in its composition: structure has been interpreted as histology rather than gross anatomy, and the book provides only limited information about the anatomical distribution of nerves. Thus description of the variations in the sympathetic outflow and its links with the sympathetic chain ('prefixing' and 'postfixing') are not included. The autonomic innervation of the eye is restricted to a discussion of two tissues only—the iris and the nictitating membrane—and there is no discussion of the eyelid. On the structural side the information is chiefly relevant to the peripheral autonomic nervous system, and details of the central nervous system are limited. So limited are they, that 'spinal cord' and 'hypothalamus' do not occur in the index and the chapter on the central nervous system (chapter 9) is only 3.5 pages long.

The description of the book explains that it is intended as a basic reference work on the autonomic nervous system for students of, and research workers in, the fields of physiology, pharmacology and neurobiology. As a description of the anatomical structures of the autonomic nervous system at a histological level, it is excellent. But it provides only limited information on the physiological mechanisms involved, and the pharmacology of the system is only briefly discussed, there being no biochemical information throughout the book. Clinical research workers will also find that it does not provide any information on pathology or the clinical relevance of any of the structures described. It may, however, be argued that this was not the intention of the book.

This book can be strongly recommended as a work of reference for any worker engaged in the study of the autonomic nervous system. It should also be available in libraries, but I feel that its information is too restricted for students or most clinical research workers.

R. H. Johnson

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Noctilucent clouds

Noctilucent Clouds. By V. A. Bronshten and N. I. Grishin. Pp. v+237. (Israel Program for Scientific Translations: Jerusalem; Wiley: Chichester, February 1976.) £8.25; $16.50.

EVER since the first observations in 1885, noctilucent clouds have been a challenge to atmospheric physicists, in particular to those dealing with problems related to the upper atmosphere. Apart from data on meteors, information on the 80-km region, where the clouds are situated, was almost nonexistent until rocket measurements became available some twenty years ago. Thus, for about seventy years, noctilucent clouds provided a playground for scientists with skill and imagination.

Noctilucent clouds also differ from most other problems in science in the following respect: during ninety years of research an enormous amount of data on various aspects of the problem has been collected and yet the two main problems remain unsolved: how do noctilucent clouds form and what do they consist of?

Since the early 1950s, V. A. Bronshten and N. I. Grishin have contributed much to our knowledge of noctilucent clouds, and their names are well known to colleagues all over the world. In view of their contact with the problem over such a long timespan, they are in the best possible position to undertake such a comprehensive presentation of the history and state of the art of noctilucent clouds.

The book is obviously meant to cover the subject of noctilucent clouds exhaustively, and in large measure the authors have succeeded in their ambitious goal. The huge amount of information on the history of theories, abandoned or still under consideration, and on observational data and techniques, classifies the book as far the most useful and comprehensive source for students at all levels. One may say that there is a bias towards citing the results of Russian scientists. For readers from Western countries, however, this is an advantage rather than a weakness, since it makes available material which is otherwise difficult to obtain.

In the reviewer's opinion the greatest value of the book is to be found in the first four of the book's six chapters: history; range of distribution and frequency of occurrence; morphology and dynamics; and luminosity. All these chapters are very comprehensive and very well presented—not surprising, since this is where the authors have their primary interest and competence. Chapter 5 on the nature of noctilucent clouds is not as complete and up to date as one might wish. The manuscript of the book was, however, finished in June 1969, and much of the information about mesospheric humidity has become available since then. The historical paper by Bates and Nicolet on water vapour in the high atmosphere clearly deserves due reference, and photochemical models with eddy transport, pointing at a humid mesopause, were also available.

This book will be a valuable reference for all those interested in noctilucent clouds.

Eigil Hesstvedt

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- ● In the review of *The Hydrolysis of Cations* (Wiley-Interscience; *Nature*, **263**, 802; 1976), the price was incorrectly quoted. This should read: $34.95; £20.85.
- ● A UK edition of *The Poverty of Power* by Barry Commoner (for review, see *Nature*, **264**, 134; 1976) has been published by Jonathan Cape, London, at £5.50.

reviews

Machiavelli with a computer

Eric Ashby

The Humane Technologist. (Science and Engineering Policy Series.) By D. Davies, T. Banfield and R. Sheahan. Pp. ix+180. (Oxford University: Oxford, 1976.) £4.75.

FOR some inscrutable reason the dust cover of this book carries a puff from the co-founders of the Club of Rome. Do not be put off by this. Any apprehension that the book was sponsored by the body which condoned a diet of exponentials for computers is soon dispelled. The authors courteously dissociate themselves from any such nonsense. They are as well equipped as anyone to compose mathematical models of future scenarios; so it is refreshing and reassuring to find them quoting, as a good example of organisational models, the table of contents of Machiavelli's *The Prince*: "and" they add, "the chapters themselves are commendable succinct runs of these models. There are, however, no rules for the construction of such models except the general one of keeping them simple and avoiding the hopeless pursuit of meaningless quantification."

The theme of Davies and his collaborators is that the technologists' honeymoon is over; society will no longer permit man to do all he is capable of doing. But technologists must not lose heart, for they hold the clues to the solution of our present perplexities. They must not lose heart but they must acquire the skills of "human perception and understanding". In brief, an education which equips the technologist to handle ideas and things should equip him, too, to handle people; the immediate threat to industrial societies is not shortage of energy or depletion of resources, it is the risk of disorder due to stresses in human relations, between management and shop floor, between government and citizen.

Drawing on a wealth of examples and the rich background of a great industry, the authors of this intelligent and sophisticated book examine the uses of models by technologists, with special reference to the circumstances of a democratic society. Enriched with historical allusions from Greek tragedy to medieval geopolitics, the book offers facts and opinions on such models as the Boston Experience Curve (which relates the cost of a product to the accumulated experience of making it); the constraints which limit the use of such models in industry and government; and the part played in technological innovation by access to investment capital. It ends by an appeal not to regard people as "inanimate thermodynamic assemblies of customers, clients, operators, and educators". Technologists have been slow to face some of the human problems they have created; yet they have (so the authors claim in their last chapter) the right sort of experience to deal with models of these problems: numeracy, familiarity with the effects of error and uncertainty, patience in reconciling concepts with the realities of everyday systems.

The book is entertaining, but the authors quite rightly want something more from the reader than "polite applause" or "stunned silence". It is a book to provoke discussion and it deserves to, for the issues it raises are critical for modern society. Perhaps the most ominous of these issues is the dilemma of what the authors call "top-down" models. Having persuaded the reader that models are important—indeed essential—to the proper deployment of technology, they then distinguish two kinds of models. On the one hand there are "bottom-up" models (they cite as an example a model for traffic flow in Westminster) which can be assembled with other subsolutions into pragmatic hierarchies to provide data for political and social decision-making; going from the particular to the general. Such models may be too empirical to provide guidance for grand strategy. On the other hand there are "top-down" models (such as the Club of Rome and the Hudson Institute indulge in) which are synoptic, and which adopt doctrinal or dogmatic guidelines. Because "bottom-up" models are liable to be difficult to integrate into a hierarchical whole, there is a temptation to adopt "top-down" models, such as the Soviet Union has used for years. Then comes the authors' warning: "a state based on top-down modelling throughout cannot afford to be democratic and must be authoritarian if it is to be effective. We reject absolute authority in politics, and so we must severely restrict the applicability of top-down modelling." But will society reject absolute authority, if the alternative on the horizon is chaos? To rescue society from having to make this choice is the challenge of this book.

For some years observers of industrial society have stressed the need for innovation in political institutions to match innovation in technology and industry. The only recent political innovation (I use the phrase deliberately) which has emerged in response to this is the multinational corporation. Do we discern in (for example) the oil companies the prototype of international power, if not yet of international government? Such corporations are deeply distrusted by the man-in-the-street; they too, like "top-down" models, are not compatible with democratic government.

The book cleverly leads the reader toward the conclusion the authors intend, namely that there ought to be a "constructive alliance" between technologists and sociologists. But how? Alas, the authors turn to education as the recipe. Education is by its very nature conservative; it is a process analogous to the transfer of genes in heredity: a sort of cultural DNA; not the place to look for such major innovation as is needed. If innovation is to come, its source is more likely to be the political philosophers. The need is for a redefinition of values and a machinery for reconciling conflicts of values. Karl Marx offered one pattern of innovation, but for those who value personal freedom the price to be paid for his pattern is too high. Is some lonely successor to Karl Marx now working in the British Museum on a political philosophy adapted to a post-industrial society? If so, he will find in this book the questions to which democratic societies seek an answer.

One postscript: the Oxford University Press used to be impeccable in its standards of proof reading. The misprints in this book are a disgrace to the Press. □

Eric Ashby (Lord Ashby of Brandon) recently retired from the Mastership of Clare College, Cambridge. He was chairman, from 1970 to 1973, of the UK Royal Commission on Environmental Pollution.

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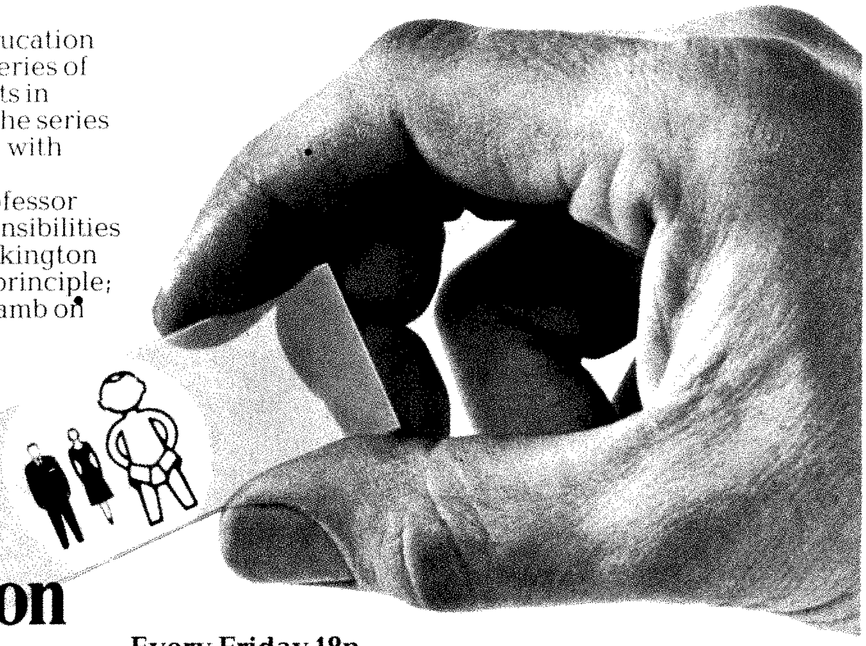
This week The Times Higher Education Supplement presents the second in a series of 8-page features covering developments in science and their social implications; the series is published quarterly in collaboration with the British Association.

This week's articles include: Professor Sir Hermann Bondi on the social responsibilities of the defence scientist; Sir Alistair Pilkington on the development of the float-glass principle; Dr B. J. Mason and Professor Hubert Lamb on climatic changes.

To anyone concerned about the role and responsibilities of the contemporary scientist, this feature makes vitally important reading. Don't miss it – in this week's



THE TIMES
Higher Education
SUPPLEMENT

Every Friday 18p

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observed in the Dead Sea region[2,3]. A continuous layer 1–2 mm thick represents a deposition of up to a million tons of dry material[3,5] (nearly pure aragonite), the derivation of which from springs would require many billions of cubic metres of water, even if very high concentrations are assumed. Such a gargantuan influx and a corresponding rise in lake levels (1 m per influx of 10^9 m^3) could not have passed unnoticed[3]. Likewise, no mud or gas volcanoes supplying CO_2 which would induce precipitation of carbonates were ever reported. For this reason, clearly stated in the previous works[2,3], the hypothesis of springs whitening the Dead Sea waters was abandoned in favour of earthquake-triggered landslides[3,4] or an aseismic chemical process[5].

Recent study of the Dead Sea[7] established that 'whitening' occurs when late summer high temperatures trigger a massive precipitation of aragonite from the hypersaline lake waters oversaturated, through evaporation, with HCO_3 and SO_4. The high temperature origin of the aragonite laminae is confirmed by their isotope composition[8]. Also the whitenings of sea waters in other parts of the world has been attributed to biogenic or chemical processes unrelated to seismicity[9,10].

(2) 15–20 White laminae were deposited[7] between 1890 and 1960. Neither these laminae, nor the conspicuous whitening of August 1959, investigated in great detail[7], can be matched with earthquake records.

(3) Composition, colour and thickness of sediments are controlled by fluctuating patterns of sediments supply, transport and deposition[11]. The pronounced lateral and vertical variations in layering and thicknesses of the dark, detrital sediments of the Dead Sea are determined by discharge, distance from river inlets, water depths and local patterns of circulation and currents[12]. In such conditions, thickness is not a unique function of time and should not be used as a yardstick in estimating time intervals.

(4) The sediment core analysed by Ben Menahem was taken in the proximal part of the Jordan delta (rough co-ordinate 199/129), where rates of deposition range up to 20 mm yr^{-1} (ref. 12), in contrast to the distal parts where values of ~ 1 mm yr^{-1} were reported[7,12]. It is very unlikely therefore, that the 170-cm long core represents the full record of 2,000 yr of deposition. Assuming the present rate of formation of white laminae, 15–20 laminae in 70 yr, it would seem that the core with its 50 white layers represents up to 300 and not 2,000 yr. Incidentally, dating and counts of laminae in the Lisan Marl, a lacustrine formation deposited in the precursor of the Dead Sea[13] between 65,000 and 15,000 yr BP indicate annual formation of white layers, implying according to Ben Menahem's supposition, a yearly occurrence of strong earthquakes . . .

(5) Last, but not least, of the objections to Ben Menahem's correlations, is the fact that the uppermost part of the discussed core is missing, having been lost in the process of coring[2]. Thus it is not clear how and why the two uppermost white layers in the core were identified as the imprints of the 1903–06 and 1834 earthquakes.

These arguments amplify and vindicate the reservations clearly stated in studies in which the possible correlation between the white laminae and earthquakes was originally discussed[2,3,5,6]. These doubts, which led to an early rejection of the hypothesis, are not mentioned in Ben Menahem's paper, which seems to rely solely on a correlation between a sequence of historic earthquakes and an undated sequence of layers in a single and incomplete, random sediment core.

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BEN MENAHEM REPLIES—Karcz, Neev and Zak have unearthed some counter-arguments[1,2], but their quoted figures for core dating, sedimentation rates, lacustrine formation dates, summer high temperatures and Lisan Marl laminae counts are of such obscure origin that all my efforts to trace their sources were fruitless.

The main issues centre, however, on "new facts" which the above authors have marshalled against my "assertions" and "assumptions". The first "fact" is the speculation[1] that "whitening" of the Dead Sea is caused by an alleged high temperature triggering of aragonite precipitation—a process that has never been duplicated elsewhere. Now, according to the statistics of Karcz *et al.*, a 'whitening' should occur every 5 yr, which renders about fifteen such events in the twentieth century alone. Unfortunately, only a single true grand-scale "whitening" was documented during this century[1], close to the occurrence of a magnitude 5 earthquake. The reported[1] "whitening" of August 1959 was much weaker and probably caused by a microtremor that passed unnoticed by the low-magnification JER station.

A second 'fact' which is mentioned twice by Karcz *et al.*, is based on the desultory observation[1]: ". . . 15 to 20 laminae were found on shore exposures and on trees which are known to have been drowned in the Dead Sea ~70 yr ago." Besides the question of relevance of this novel 'tree-meter' to our mud-core deposits, this observation[1] implies a repetition of 'whitening' on the average every 3 yr since 1890. Ashbel[5] on the other hand, counted 2,000 annual grey layers in the mud core of Elazari Volcani[6].

Another "fact" presented by Karcz *et al.*, which contradicts the published reports of Amiran[7] and Ashbel[5], is their dubious statement : "No pre-earthquake emergence of new springs was observed in the Dead Sea region". It has, however, been recently established[8] that the Dead Sea is sitting on a huge active fault, some 60-km long, with an estimated area of 1,500 km^2. Its motion, whether abrupt or slow, can and should affect the regular flow of the many springs inside and on the shores of the eastern side of the lake. It may, in addition, cause massive landslides and other topographical changes at the flanks and bottom of the Dead Sea, as already suggested by Shalem[9].

The exact mechanism by which the fault slip or pre-earthquake creep is coupled to the outbreak of turbidity is immaterial to the correlation under discussion.

There is nothing therefore in the objections of Karcz *et al.* that has bearing on the remarkable correlation[10] of past seismic events to the mud-core white layers. Karcz *et al.* have not added new data or information to the already known reports[3-7,9] while their use of the "drowned-tree timescale"[1] and the "whitening" speculation[1] leads them to predict an almost everlasting snow-white colour for the Dead Sea.

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matters arising

Brain tryptophan in rats on a high fat diet

Inasmuch as the article by Hutson *et al.*[1] constitutes a serious criticism of the data reported from our laboratories over the past three years[2–6], we would like to make the following reply:

- In 1974, some of us[4] examined the validity of the various methods then available for measuring free tryptophan in blood. We described fully that the equilibrium dialysis method, performed at 37 or at 0 °C, gave identical results for serum-free tryptophan and for serum NEFA levels. The values for NEFA were the same as those measured before dialysis; this test was done with sera containing high (1 meq l^{-1}) and low (0.2 meq l^{-1}) amounts of NEFA. We thus ruled out the potential problem of lipolysis during dialysis over two years ago, as Hutson *et al.* admit only parenthetically (page 143).
- In this same paper[4], we tested the method of Knott, Curzon, and their colleagues, which involves separation of free tryptophan using Amicon filter cones centrifuged at 800*g*. In this method, no attempt is made to control $p\mathrm{CO}_2$, and thus serum or plasma *p*H, during dialysis. Tryptophan binding is extremely sensitive to *p*H changes; using this method, the *p*H can run as high as 8.0 or more, as observed by Madras *et al.*[4]. We note that in the article by Hutson *et al.*[1], on page 143, *p*H values of 7.75, 7.78, and higher were recorded, which affirms the non-control of *p*H in this method, and confirms the findings of Madras *et al.*[4].
- Hutson *et al.*[1] claim that they obtain similar findings when they assay either plasma or serum. But they fail to provide data on free tryptophan values in serum; instead they give data for serum UFA—and assume that free tryptophan behaves similarly. This assumption must be proved in each new experimental situation.
- Finally, we would like to note the following: (1) Hutson *et al.*[1] provide little information about the diets consumed by their animals. What were the protein and fat contents of these diets? How can the authors call the standard laboratory chow diet a "control diet?" (2) How much food was consumed by each group? (3) Why was plasma total tryptophan lower in the group eating the high fat diet? It seems to us that this "diet" experiment is uninterpretable as it is presented.

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Hutson, Knott and Curzon reply—We fail to see that the essential point of our letter is seriously affected by any of the issues raised by Fernstrom, Munro and Wurtman. They refer to the work of Madras *et al.*[2] in which rats on a high-fat diet apparently had high serum free tryptophan not associated with high brain tryptophan. Our own results lead us to question the physiological relevance of these findings as large amounts of unesterified fatty acids (UFA) can be formed by plasma or serum of fat-fed rats *in vitro* and this may lead to the liberation of tryptophan from albumin after blood collection.

Taking the points raised in their letter in order:

- In quoting the finding of Madras *et al.*[2] that UFA did not increase on dialysing serum at 37 °C our use of the grammatical convenience of parentheses was not intended in any way as a criticism of the validity of this result. However our own findings[1] suggest that UFA may have already increased during the preparation of serum from fat-fed rats.
- As already explained[1] the small *p*H differences between plasmas of control and fat-fed rats would have had negligible effects on free tryptophan concentration.
- It would be most surprising if UFA changes did not cause free tryptophan changes in rat serum as in plasma. We find a significant positive correlation ($P<0.01$, $n=16$) between percentage free tryptophan and UFA of human sera.
- We did not provide detailed information on the food presented to the rats or on the amount they ate, because this had little relevance to our main conclusion—that changes occur *in vitro* in rat plasma and serum taken from fat-fed rats.

In the circumstances, and considering the many findings consistent with free tryptophan influencing brain tryptophan (refs 3–8 are to papers published in 1976) the results of Madras *et al.*[2] can hardly be interpreted with confidence.

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Dating earthquakes by mud profiles of lake sediments

Ben Menahem[1] asserts that the white laminae in the recent sediments of the Dead Sea represent past occurrences of earthquakes, and identifies in a single sediment core all major shocks of the past 2,000 yr. This exhumation of a long abandoned concept[2–6] relies on two fallacious assumptions. First, new springs are assumed to open up in the Dead Sea before an earthquake, and discharge a 'white material' which whitens the lake waters and deposits on the bottom a lamina of a thickness proportional to the earthquake magnitude. Second, the described 170-cm long core from the proximal part of the Jordan delta is assumed to represent the full record of deposition in the past 2,000 yr, and thicknesses of the dark-coloured sequences sandwiched between successive white laminae are assumed to be a measure of earthquake recurrence times[2,3,6].

Both these assumptions are incompatible with facts:

(1) No pre-earthquake emergence of new springs nor large fluctuations of flow from existing springs have been

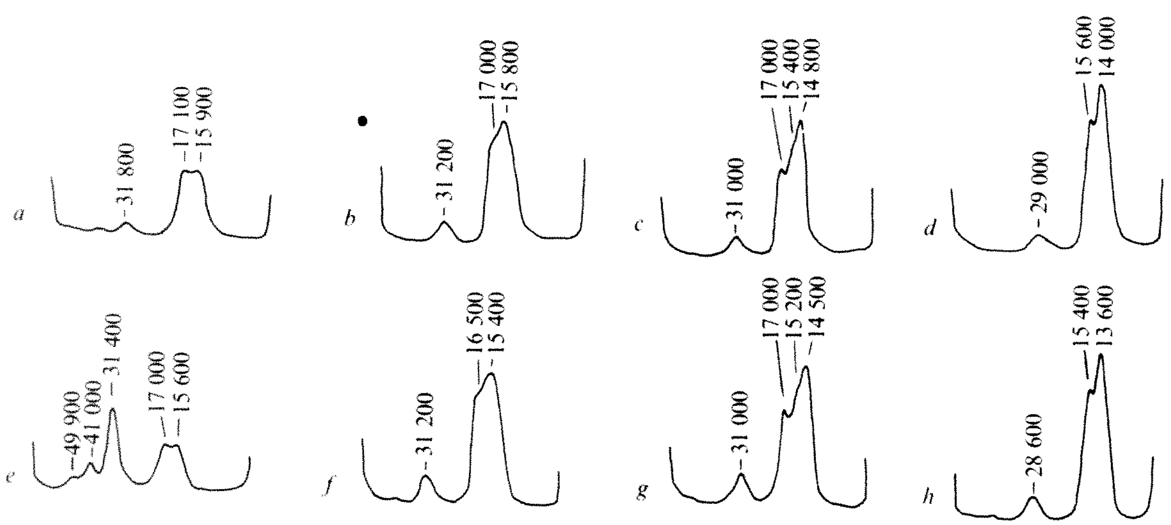

Fig. 2 Polyacrylamide gel electrophoresis (PAGE), in the presence (*a*–*d*) and in the absence (*e*–*h*) of reducing agent, of Fraction A_1 prepared by trypsin digestion of reduced wool in various conditions. *a*, 0 °C, 30 min, w/e (wool–enzyme ratio) 250:1; *b*, 0 °C, 60 min, w/e 50:1; *c*, 20 °C, 60 min, w/e 50:1; *d* 40 °C, 60 min, w/e 50:1; *e*, 0 °C, 30 min, w/e 250:1; *f*, 0 °C, 60 min, w/e 50:1; *g*, 20 °C, 60 min, w/e 50:1; *h*, 40 °C, 60 min, w/e 50:1. *a*–*d*, The most concentrated A_1 fraction from each chromatograph in Fig 1*a*–*d* was diluted to 0.2 mg ml^{-1} with 0.1 M Na_2HPO_4 buffer at *p*H 8.0, mixed with an equal volume of 8 M urea, 2% sodium dodecyl sulphate (SDS), 2% mercaptoethanol (ME) in distilled water and heated to 45 °C for 1 h. After cooling, one drop of the solution was applied to a 10% polyacrylamide gel cross-linked with 0.6% *bis*-acrylamide. After electrophoresis in 0.1% SDS, 0.1 M Na_2HPO_4, *p*H 8.0, the gels were stained overnight with 0.25% Coomassie brilliant blue dissolved in 20% trichloroacetic acid, destained in a mixture of 3 volumes acetic acid, 2 volumes methanol, 35 volumes water, and scanned with a Joyce–Loebl densitometer. Bovine serum albumin, pepsin, trypsin, myoglobin, haemoglobin, lysozyme and chymotrypsin were denatured in the same way and run at the same time as the A_1 fractions. The molecular weights of the bands from the A_1 fractions were estimated by interpolation on the line obtained when log (molecular weight) of the standards was plotted against the mobility during electrophoresis. *e*–*h*, The most concentrated fractions from Fig 1*a*–*d* were denatured as described above except that ME was omitted. The molecular weights of the component chains of A_1 were estimated as before, except that the standard proteins were also denatured without reducing agent.

a large peptide would account for the 41,000 molecular weight band in Fig. 1*e*.

The molecular weight of fraction A_1, prepared by trypsin digestion of reduced wool at 20 °C for 60 min with a 50:1 wool–trypsin ratio (A_1^{20}), is 50,000 to 60,000 measured at several *p*H values in the ultracentrifuge, using the Ehrenberg method; or 53,600 calculated from the sedimentation coefficient (3.22 s), the diffusion coefficient (4.83×10^{-7} cm^2 s^{-1}) and the partial specific volume (0.69 ml g^{-1}) at *p*H 6.0 using the Svedberg equation[6]. It is not possible to say definitely whether A_1^{20} has three or four component chains when these molecular weights of undissociated A_1^{20} are compared with the molecular weights of the component chains from denatured A_1^{20} Fig. 2*c* and *g* show that the proteins from A_1^{20} responsible measurements. Three is more likely because the molecular weights of undenatured A_1^{20} may be high because of aggregation in solution[8].

Assuming that the staining of each band is approximately proportional to the amount of protein in the band, Fig. 2*c* and *g* show that the proteins from A_1^{20} responsible for the two major peaks and the shoulder between them, are not present in equal amounts. Also, all the molecules in one PAGE band do not necessarily have the same composition. Therefore, each undenatured molecule may be composed of three chains, each slightly different in weight. The chain weights (and compositions) are not necessarily the same in different undenatured A_1^{20} molecules. This heterogeneity is an expected result of the limited proteolysis method of preparation.

The peptide bonds involving the carboxylic acid groups of the 26 lysine and 31 arginine residues in each undenatured A_1^{20} molecule[6] are not hydrolysed by trypsin during the preparation. This implies that the component chains of A_1^{20} are held in a rigid conformation throughout most of their length by hydrogen bonds and other secondary valence forces, so that the complexes which are necessary for hydrolysis to occur cannot form between trypsin molecules and the chains. The low yield of fraction A_1 (7.5% of the weight of the wool)[14] implies that, after reduction, the chains in the remainder of the microfibril component macromolecule may be more flexible and therefore digested to small peptides by trypsin. The role of the rigid part of the macromolecule may be to interact with the rigid parts of similar, neighbouring macromolecules to ensure their correct alignment and staggering in the microfibril, and experiments are in progress to investigate the aggregation properties of A_1. The flexible part of the macromolecule may be partly responsible for the mechanical resilience of the fibre, and it may interact with the high-sulphur proteins of the fibre matrix[5].

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main bands, component 7 (molecular weight 46,000) and component 8 (molecular weight 51,000)[3,4], and it has been suggested that the microfibrils are composed of regularly arranged macromolecules (molecular weight 143,000) consisting of two component 7 molecules and one component 8 molecule[5].

Merokeratin A_1 is a rod-shaped, low-sulphur protein fragment (dimensions ~2×21 nm, molecular weight 50,000 to 60,000, 64%, α helix) which dissolves when reduced wool is treated with trypsin[6]. In the experiments described here, A_1 has been prepared in a variety of conditions and dissociated into its component chains using urea, sodium dodecyl sulphate (SDS) and, in some experiments, mercaptoethanol. The chain molecular weights have been measured by polyacrylamide gel electrophoresis (PAGE) and compared with the molecular weight of undissociated A_1. The results imply that A_1 consists of three protein chains, which suggests that it may be part, approximately one third of the length, of the microfibrillar macromolecular component protein.

Similar merokeratins have been prepared from low-sulphur wool proteins[7–9] and from epidermal keratin proteins[10–13] by protease digestion.

Different amounts of trypsin and different temperatures and times of digestion were used in four methods of treating reduced wool (Fig 1*a*–*d*). The wool debris was removed by centrifugation and the supernatant protein solutions were exposed to the air to reoxidise the thiol groups to disulphide bonds: the solutions were first diluted to favour the formation of intramolecular, interchain crosslinks[14]. Each solution was acidified to precipitate the protein. When the precipitates were redissolved in *p*H 8.0 buffer and fractionated on a Sepharose 6B column, the elution volume of the major retarded peak, fraction A_1, was independent of the severity of the trypsin treatment. The yield of A_1 was lower when the mildest conditions were used for the digestion (Fig. 1*a*). A small amount of protein (V) is eluted at the void volume of the column. Fraction B* is a heterogeneous mixture of low-sulphur peptides crosslinked by disulphide bonds: its composition varies with the method of preparation (S.S.L., unpublished results).

The most concentrated A_1 fraction from each of the Sepharose fractionations in Fig. 1 was denatured with urea and SDS in the presence and in the absence of mercaptoethanol. PAGE showed either two or three intensely stained, partially resolved bands, and one or more less intense bands (Fig. 2). Standard proteins were denatured in the same way and run in parallel to calibrate the gels. The molecular weights of the main bands were between 14,000 and 17,000. The proteins from the more severe trypsin digestions were slightly shorter than those from the less severe digestions. The calculated values of the molecular weights in Fig. 2 are probably within ± 10% of the true values[15].

All the gels showed a band of protein which migrated more slowly than the main bands, which had a molecular weight of about 31,000, or about 29,000 when digestion was at 40 °C. The band was most prominent when A_1 was prepared by the mildest digestion procedure and denatured with no reducing agent present (Fig. 2*e*). Its susceptibility to reducing agent (see Fig. 2*a*) suggests that the protein of the band may consist of two proteins with molecular weights between 14,000 and 17,000 crosslinked by one or more disulphide bonds. The lower intensity of the 31,000 or 29,000 bands when digestion was carried out under more severe conditions (Fig. 2*f*–*h*) suggests that the crosslinks are in the ends of the molecules prepared by mild digestion, and that these ends are removed in the more severe digestions. A weakly stained 31,000 or 29,000 band persists even when A_1 is denatured with a reducing agent present (Fig 2*a*–*d*). This could be explained if some cystine residues were converted to non-reducible lanthionine or lysinoalanine residues during the alkaline reduction of wool before the trypsin treatment[16,17]. The small peak of protein with a molecular weight of 49,900 in Fig. 2*e* suggests that after the mildest digestion there may be a small amount of material in which three protein chains with molecular weights between 14,000 and 17,000 are crosslinked. Two of these proteins crosslinked together and to

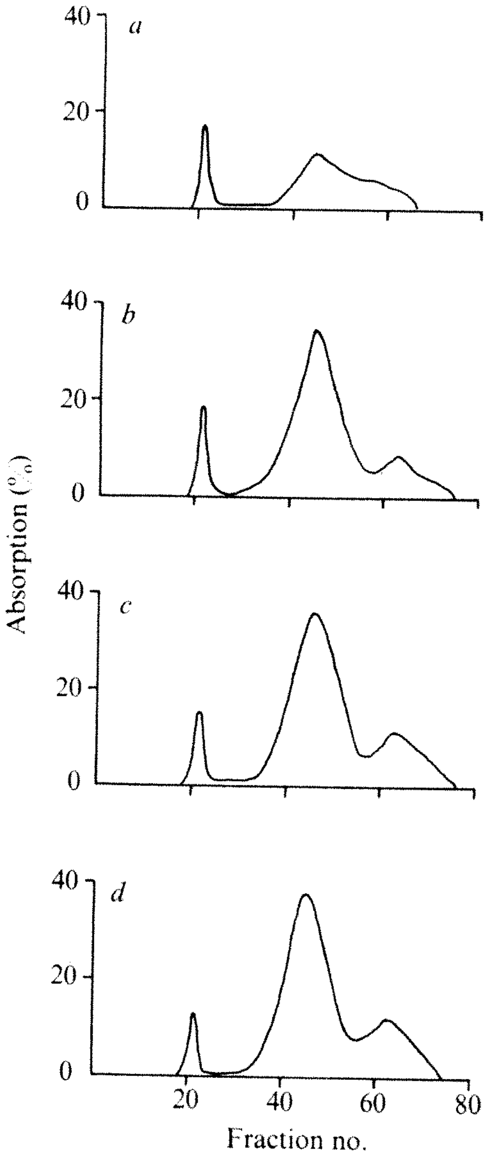

Fig. 1 Sepharose 6B chromatography of protein fragments dissolved from reduced wool by trypsin under various conditions. 2.0 g samples of purified Lincoln wool were reduced for 18 h at room temperature in 1.0 l of 0.1 M Na_2HPO_4, 0.2 M thioglycollic acid adjusted to *p*H 10, rinsed four times with 100 ml 0.1 M Na_2HPO_4 at *p*H 8.0, transferred to 40 ml of the *p*H 8.0 buffer, and treated with trypsin. Temperatures, times of digestion and wool–enzyme ratios (w/e) were as follows: *a*, 0 °C, 30 min, w/e 250:1. *b*, 0 °C, 60 min, w/e 50:1. *c*, 20 °C, 60 min, w/e 50:1. *d*, 40 °C, 60 min, w/e 50:1. After the digestions the trypsin was inhibited by 8 ml 0.25% phenylmethylsulphonylfluoride[18] in 50% v/v isopropanol–water. The digests were centrifuged at 50,000 *g* for 30 min. The supernatants were diluted to 1.5 l with 0.1 M Na_2HPO_4, *p*H 6.0, and exposed to the air in shallow dishes for 22 h at room temperature[14]. In each experiment, the reoxidised protein was precipitated by adjusting the *p*H to 4.8. The precipitates were collected by centrifugation at 1,000 *g* for 10 min, and redissolved in 5 ml 0.1 M Na_2HPO_4 *p*H 8.0 buffer. The solutions were dialysed for 2 h against the same buffer, clarified at 150,000 *g* for 10 min, applied in turn to the column, and eluted with the *p*H 8.0 buffer. Fraction A_1 is the major retarded peak. V and B* elute at fractions 21 and 62 respectively. The percentage absorption of the eluent from the column was measured at 254 nm in an LKB Uvicord I.

factor which reflects the decay of the population of the absorbing species being viewed. By defining a function $R(t) = (A(t)_\parallel - A(t)_\perp)/(A(t)_\parallel + 2A(t)_\perp)$ which is analogous to anisotropy commonly used for fluorescence polarisation[14], a purely exponential expression for dichroic decay is obtained.

$$R(t) = 3/10\alpha\beta \exp(\theta t) \qquad (3)$$

This is independent of the decay kinetics of the phototransient.

As reported previously[9], little decay in dichroism can be detected at room temperature in aqueous suspensions of purple membrane during the lifetime of $M(410)$. Transient lifetimes, however, increase markedly with decreasing temperature ($Q_{10} \approx 3$) and adequate first-order plots were obtained (s.d. $< 10\%$) consistent with equation (3). The results are presented in Fig. 2(A) in the form of an Arrhenius plot of the viscosity dependent ratio T/θ. The best straight line yields an energy of activation for microviscosity, E, of 7.4 ± 0.3 (s.d.) kcalorie mol^{-1}. This is significantly higher than that for water[15], and shows the presence of rotational freedom in addition to that of fragment tumbling. Purple membrane, when treated with the protein crosslinking reagent glutaraldehyde, continues to undergo reversible photochemical cycling. The lifetimes are slightly increased, facilitating a more extended Arrhenius plot. This (see Fig. 2(B)) yields $E = 4.4 \pm 0.04$ (s.d.) kcalorie mol^{-1}, consistent with published values for water[15]. The size calculated[16] for the membrane fragments (diameter $= 0.5$ μm) assuming them to simulate oblate ellipsoids is in excellent agreement with published values (diameter $= 0.5$ μm)[1], affording a test for the validity of the theory.

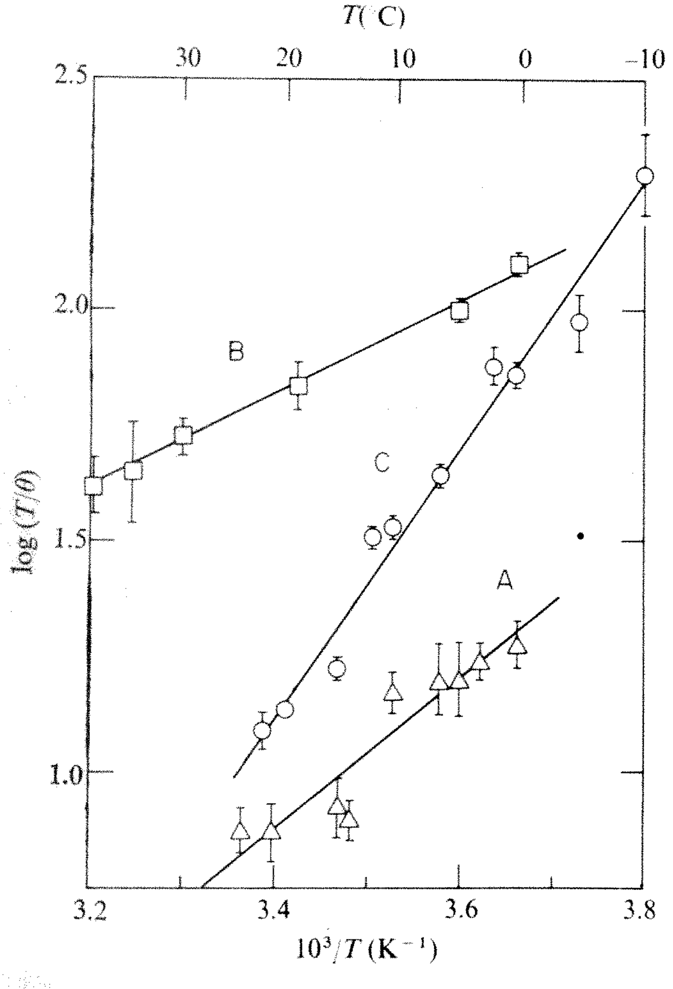

Fig. 2 Arrhenius plots of the viscosity-proportional function T/θ. Bars represent standard deviation in the measurement of θ. △, Membrane fragments in water; □, membrane fragments pretreated with glutaraldehyde and suspended in water; ○, membrane fragments in a 20% polyacrylamide gel.

Both untreated and glutaraldehyde-fixed purple membranes were incorporated into a 20% polyacrylamide gel by *in situ* polymerisation of acrylamide monomer added to aqueous purple membrane suspensions. At this concentration the membrane fragments may be expected to be completely immobilised. The photochemistry seems unchanged, with phototransient yields and lifetimes in the polymer indistinguishable from those in pure water. For the glutaraldehyde-fixed sample no dichroic relaxation was detectable within the whole temperature range (detection limits at 20 and 0 °C, respectively, $\theta = 10^{-1}$ and 10^{-2} s^{-1}). But for the unfixed polymer first-order dichroic decay was observed, yielding $E = 13 \pm 0.05$ (s.d.) kcalorie mol^{-1} (Fig. 2(C)).

The results presented here show that the chromophore of bacteriorhodopsin is not totally immobilised within the purple membrane. The significant rotational rate constants found, 20 s^{-1} at room temperature, are too high to be attributable to rotation of individual protein molecules *in toto* within the crystal lattice, and must indicate internal conformational change. It is tempting to speculate, based on the close coincidence of E for rotational mobility and that found for the formation of $M(410)$ (12 kcalorie mol^{-1})[12], that the deprotonation step which produces this intermediate is controlled by intramolecular movement involving the retinylidene group (which simulates Brownian motion). The establishment of internal mobility within bacteriorhodopsin suggests that it might be profitable to look for an analogous process, in contrast to the criticised tumbling mechanism, in the transmission of the visual signal in rhodopsin subsequent to the primary *cis–trans* isomerisation.

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Three-chain merokeratin from wool may be a fragment of the microfibril component macromolecule

WOOL fibres consist mainly of long cortical cells filled with microfibrils, 7–8 nm diameter, embedded in a matrix. The microfibrils are composed of proteins with a lower sulphur content and a higher α-helix content than wool; the matrix proteins have a higher sulphur content than wool and no α helix[1,2]. When reduced and carboxymethylated low-sulphur proteins from wool (SCMKA) are separated by starch gel electrophoresis, there are two

Selectivity coefficients were calculated from the data. The average value of S_1 ^{3}H (for animals fed separate food sources) was 0.2 (±0.1) while the average S_2 ^{3}H value (for animals fed mixed food) was 0.5 (±0.1).

Lampert[7], using an exposure time of 15 min and mixed food only, showed that young daphnids are remarkably selective in their feeding habits. Table 2 shows that feeding rate is highest during the shortest exposure times and lower during longer exposure times. In all cases, however, no selectivity was found.

There are two possible reasons for this difference: (1) *Daphnia* may digest bacteria faster than algae, or (2) the accumulation, in long-term experiments, of bacterial material as opposed to algal material may be due to the shorter retention time of ^{14}C-labelled substances (algal source), which might be used for intensive metabolic activities such as respiration. The results presented here and derived from long-term experiments with *Ceriodaphnia*[6] and *Daphnia*[7] suggest the following processes. Initially ^{14}C-labelled algal materials are ingested in larger amounts during normal feeding. Later, the rapid digestion of bacterial materials results in the accumulation of ^{3}H-labelled substances. I conclude that the rate of ingestion is influenced by the size of the particles present in natural or artificial bodies of water. This is important in ecosystems containing suspended particles of a diversity of sizes.

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Chromophore mobility in bacteriorhodopsin

THE sole protein found in the purple membrane of *Halobacterium halobium* contains retinal covalently bound by means of a protonated Schiff base linkage to lysine[1,2]. The only established function of this protein is to act as a light-driven proton pump producing a transmembrane proton gradient, which is coupled to ATP synthesis in the living organism[2,3]. In so far as the retinal prosthetic group resembles that found in the visual pigment of vertebrates, rhodopsin, the name bacteriorhodopsin has been widely adopted for this protein[3]. Considerable work has been done on the biochemistry and photochemistry of the purple membrane, both for its intrinsic interest as a unique photosynthetic membrane and with respect to the identification of possible mechanistic relationships between its photochemical cycle and that of the visual process. We report here a study of the later stages of the photochemical cycle by flash photometry with plane polarised light. Our results indicate conformational change in the bacteriorhodopsin molecule at the site of the visible chromophore.

There has been speculation as to whether rotational motion of the visual pigment is an essential link in the signal chain in the visual process[4]. It has been demonstrated that rhodopsin molecules undergo rapid Brownian rotation within the membrane[4,5] and the theory has been proposed (but criticised on energetic grounds[6]) that rotation about an axis parallel to the plane of the rod disks ('tumbling') could enable rhodopsin to act as a transmembrane ion carrier[4]. In contrast, bacteriorhodopsin exists in a crystalline form in the purple membrane[7]. Consequently, Brownian diffusion of individual molecules would be expected to be severely restricted, and this has been confirmed[8,9]. But during a study[10] of the temperature dependence of the photochemical cycle in aqueous suspensions we were surprised that the measured rotational relaxation times gave an activation energy for the medium viscosity (see below) which differed significantly from that characteristic of water. If the protein chromophore is rigidly bound within a crystal lattice which has dimensions comparable with those of the membrane, then rotational relaxation would be exclusively a consequence of the tumbling of the individual fragments, constrained only by the viscosity of the water in which they were suspended. This consideration induced us to carry out a more extensive study of rotational mobility.

The bacteriorhodopsin photochemical cycle contains a sequence of phototransients of which one, *M*(410), has been shown to have the retinal Schiff base unprotonated[11]. The decay of *M*(410) is linked kinetically to the reappearance of the ground state *BR*(570), with a lifetime at room temperature in the millisecond region[12]. Rotational mobility was investigated by measurement of the time dependence of the decay of linear dichroism of the absorption band of *M*(410) at 410 nm. By means of a conventional flash photometer[9], purple membrane samples were excited with μs light flashes filtered successively through a 500-nm cutoff filter and a linear polariser with its optical axis vertical (laboratory *a* axis, Fig. 1). Light transmission was then viewed in the *c* direction with a beam polarised alternately parallel and perpendicular to the exciting light. The time dependence for absorption of the two polarised analysing beams is given by[13]

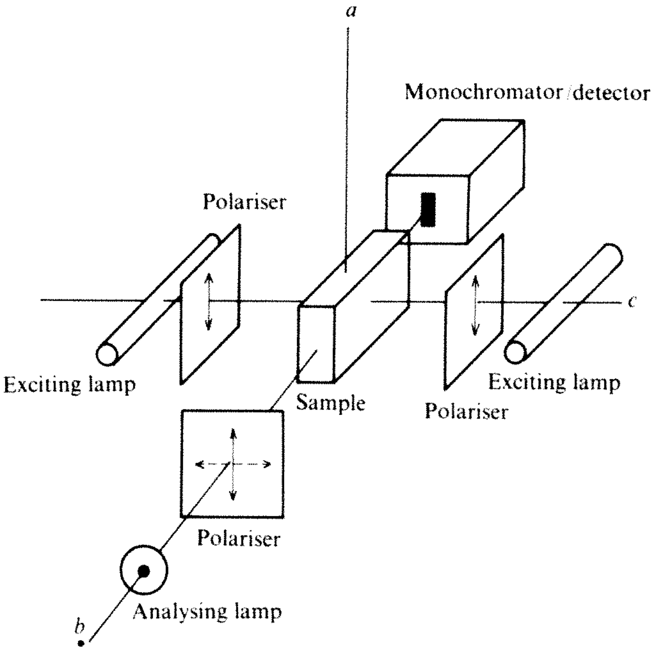

Fig. 1 Optical layout of flash photometer for measurement of relaxation of linear dichroism in phototransient absorption.

$$A(t)_{\parallel} = 5/3 - \alpha\beta\exp(\theta t) \qquad (1)$$

$$A(t)_{\perp} = 5/3 - \tfrac{1}{2}\alpha\beta\exp(\theta t) \qquad (2)$$

where θ is the rotational rate constant, proportional to temperature and inversely proportional to medium viscosity and shape and size of the rotating species ($kT/V(\mathrm{r})\eta$), β is a function of the optical transition moments of the chromophore absorbing the exciting light and the species being viewed (*M*(410)), and α is a randomisation factor ($\alpha < 1$) which encompasses instrumental artifacts in addition to unidentified (non-Brownian) causes of depolarisation[13]. Total time-dependent absorption is then given by equations (1) and (2) multiplied by a weighting

Table 1 Content of food source, suspension and incubation times

No.	Bacteria Cells per ml (× 10^{7})	Cells per c.p.m.	Algae Cells per ml (× 10^{5})	Cells per c.p.m.	Incubation times
1	5.0	172	13.7	22	0.5; 2; 5; 10;
2	7.5	490	5.7	1	0.3; 1; 2; 5;
3	2.5	1,306	15.9	31	0.3; 1; 1.5; 2.5; 5;
4	6.0	530	10.4	10	5; 10; 20; 40; 60;
5	5.0	903	7.2	10	5; 10; 20; 40; 60;

the feeding mechanism of *Daphnia magna* (Strauss) during short exposure to bacteria, algae or a mixture of the two.

Chlamydomonas reinhardti (Dangeard), cultured in Bristol's medium and labelled with ^{14}C (ref. 6) and *Chlorobium phaeobacteroides* Pfennig cultured on Pfennig's medium and labelled with ^{3}H (ref. 6) served as food sources. They were collected and resuspended in filtered lake water and 0.3 ml of each suspension was solubilised in 5 ml of Aquasol Universal LSC cocktail, and radioactivity was determined in a Packard liquid scintillation counter. The cell concentration of the suspension was determined by counting the algae on a haemocytometer and by measuring the specific absorbance of bacterial chlorophyll at 715 nm. The correlation between absorbance at 715 nm and cell number was obtained by incubating serial dilutions of the cell culture in Agar-shake tubes containing Pfennig's medium and counting the colonies that developed after 3 weeks. By this method an absorbance of 1 was equivalent to 4.35×10^{7} cells per ml (B. Z. Cavari, unpublished results).

The volume of cells was measured and their wet weight was calculated by assuming a density of 1.0. Values of 1.5×10^{-4} μg per cell and 1.6×10^{-8} μg per cell were found for *Chlamydomonas* and *Chlorobium*, respectively.

Several hundred newborn daphnids were pipetted from the culture into filtered lake water. They were starved for 24 h in the dark at room temperature (22–24 °C). Each experiment was carried out as follows: ten animals were introduced into tubes containing algae, bacteria or mixtures of both and incubated for different times (Tables 1 and 2). All experiments were done at room temperature in the light. With exposure for more than 5 min, the test tubes were mixed gently every 5–10 min. At the end of the exposure, the daphnids were removed as quickly as possible by sieving through a nylon net and washed with filtered lake water. They were then transferred into scintillation vials and 5 ml of Aquasol was added. After 24 h, radioactivity was determined. The samples were then sonicated in an ultrasonicator (MSE) at high power, in ice for 2 min. This treatment disintegrated the organisms. Counts after sonication were higher by a factor of 1.5 (±0.2) for ^{14}C and a factor of 1.1 (±0.1) for ^{3}H. Therefore all samples were sonicated before counting.

The ingested biomass is shown in Table 2. The average bacterial biomass ingested in each experiment was 1.3 (±0.3) times greater when the animals were fed on mixed food compared with bacteria alone. In contrast, the ratio between ingested biomass of algae by the daphnids that were fed mixed food compared with those fed algae alone was 0.5 (±0.1).

It is likely that in the presence of *Chlorobium* alone the rejection rate is higher than in the presence of the relatively large algal cells. Conversely the presence of bacterial cells in the mixed food source lowered the ingested biomass of *Chlamydomonas*. As Table 2 shows when fed bacteria alone, the daphnids ingested $0.1–4.2\times10^{-3}$ μg per animal, and $0.1–5.7\times10^{-3}$ μg per animal when exposed to mixed food. The similar ranges of ingestion of algae were 0.1–12.3 μg per animal when the daphnids were fed on algae, and 0.1–10.7 μg per animal when given mixed food.

Table 2 Ingested fresh-weight in μg per ind and selectivity coefficients (see text)

No.	Incubation time (min)	Bacteria 10^{-3} μg per ind	Algae μg per ind	Bacteria and Algae Bacteria 10^{-3} μg per ind	Algae μg per ind	$S_1{}^3H$*	$S_2{}^3H$*
1	0.5	0.3	0.6	0.3	1.6	1.6	1.6
	2	0.2	1.9	0.1	0.2	0.3	1.5
	5	0.4	5.9	0.2	1.2	0.2	0.6
	10	0.6	4.3	0.2	2.3	0.4	0.3
Average		0.4	3.2	0.2	1.3		
2	0.3	0.5	0.3	0.4	0.1	0.1	0.3
	1	0.2	0.2	0.4	0.1	0.1	0.3
	2	0.3	0.1	0.9	0.1	0.2	0.3
	5	0.3	0.2	0.5	0.1	0.1	0.4
Average		0.3	0.2	0.6	0.1		
3	0.3	2.2	5.6	2.9	4.1	0.1	0.3
	1	2.5	5.6	5.1	3.1	0.2	0.6
	1.5	4.2	8.2	2.6	3.9	0.2	0.3
	2.5	2.0	8.5	5.1	10.7	0.1	0.2
	5	3.5	12.3	5.7	5.9	0.1	0.4
Average		2.9	8.0	4.3	5.5		
4	5	0.9	1.8	3.4	0.5	0.1	0.9
	10	2.2	0.7	1.6	0.8	0.5	0.3
	20	1.2	1.9	2.3	1.2	0.1	0.3
	40	1.0	1.1	2.6	0.8	0.1	0.4
	60	2.2	1.6	2.0	0.7	0.2	0.4
Average		1.5	1.4	2.4	0.8		
5	5	0.4	0.4	0.3	0.1	0.1	0.6
	10	0.4	0.2	0.3	0.1	0.2	0.6
	20	0.4	0.3	0.4	0.2	0.1	0.3
	40	0.4	0.3	0.5	0.1	0.2	0.5
	60	0.1	0.4	0.1	0.1	0.04	0.3
Average		0.3	0.3	0.3	0.1		
Total average						0.2	0.5
± standard deviations						0.1	0.1

ind, Individual.
* Selective coefficients.

Table 1 Effect of SHAM on the motility of bloodstream form *T. brucei* incubated in a glucose–glycerol mixture

Substrate present (mM)		SHAM added	Motility after		
Glucose	Glycerol	(1 mM)	5 min	15 min	30 min
25	0	—	++++	+++	+++
25	0	+	+++	+++	++
25	0.5	+	+++	+++	++
25	1.0	+	+++	++	+
25	2.0	+	++	+	±
25	5.0	+	+	—	—
25	10.0	+	+	—	—

Heparinised rat blood infected with *T. brucei* EATRO 427 was diluted 1:1 with 50 mM phosphate buffer, *p*H 8.0, containing 45 mM NaCl and 50 mM glucose. Samples (1 ml) were incubated at room temperature with various amounts of glycerol plus SHAM. Samples were taken at the times indicated and examined by phase contrast microscopy within 30 s for motility.

pathway for the reoxidation of NADH, which allows the synthesis of 1 mol of ATP per mol of glucose when the glycerolphosphate oxidase is blocked[9,10]. An inhibitor of this pathway, combined with SHAM, might therefore be lethal to the trypanosome, though either inhibitor alone would be ineffective[10]. We have found that SHAM and glycerol represent such a lethal combination. We were led to these experiments by previous work by Ryley[11], who has shown that glycerol inhibits glucose utilisation by trypanosomes in anaerobic conditions.

Table 1 shows that SHAM alone does not abolish motility of trypanosomes incubated in blood with glucose as substrate, but that motility progressively decreases when increasing amounts of glycerol are included in the mixture. In other experiments with purified trypanosomes glycerol concentrations as low as 1 mM were sufficient to immobilise the trypanosomes. A more quantitative analysis of the effects of glycerol is given in Fig. 2. In this experiment the rate of glycolysis was monitored by measuring pyruvate production. Glycerol has little effect on glycolysis in the absence of SHAM, but in the presence of SHAM 5 mM glycerol is sufficient to inhibit pyruvate formation by 99%. Half-maximal inhibition was obtained with 0.8 mM glycerol (0.4 and 1.2 in two other experiments). This inhibition by glycerol can be explained by product inhibition of the putative alternative pathway for NADH reoxidation[9,10], but other explanations remain to be excluded.

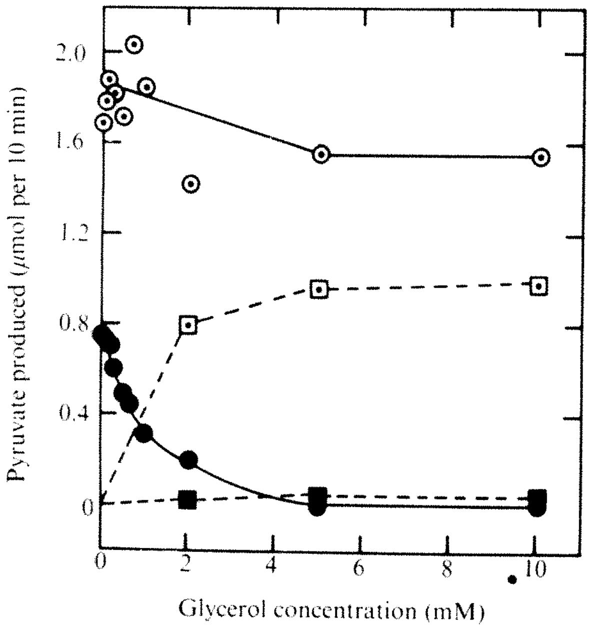

Fig. 2 Effect of SHAM on pyruvate production by *T. brucei* incubated in glucose–glycerol mixtures. Purified trypanosomes (0.7 mg of protein) were incubated at 37 °C in a 1-ml reaction mixture containing 50 mM sodium phosphate buffer, 45 mM NaCl, *p*H 8.0, with glucose and/or glycerol as substrate. SHAM was added as a solution in dimethylformamide; an equal volume of dimethylformamide was added to the control incubations. Samples were deproteinised with perchloric acid at zero time (to correct for any pyruvate in the trypanosomal suspension) and after 10 min of incubation. After neutralisation the supernatants were assayed for pyruvate. Further details of the methods used are given in ref. 9. Circles, trypanosomes incubated with 10 mM glucose plus 0–10 mM glycerol; squares, trypanosomes incubated in glycerol alone. Open symbols, no SHAM added; closed symbols, plus 1 mM SHAM.

As it is possible to obtain plasma SHAM levels that completely block the glycerolphosphate oxidase[10] and glycerol levels exceeding 5 mM (ref. 12), our results predict that it should be possible to kill *T. brucei* also in the living rat with SHAM+glycerol. This prediction has been verified[13]. Glycerol administration might also potentiate the therapeutic action of suramin, a drug used in the treatment of human sleeping sickness, as the glycerolphosphate oxidase system is the only trypanosomal enzyme reported to be inhibited by low suramin concentrations[14]. Finally, our results show how insight into trypanosome metabolism could lead to the development of drug combinations that would not have been detected in a conventional screen for trypanocidal drugs, because neither of the drugs alone has measurable trypanocidal action. It might be useful to extend present drug screening procedures to include screening of drugs in the presence of SHAM or glycerol.

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Feeding of *Daphnia* on *Chlamydomonas* and *Chlorobium*

AMONG investigations of filtration mechanism and food selectivity of filter feeders[1–7], Gophen *et al.*[6] have used double labelling to show the preferential absorption of bacteria by *Ceriodaphnia reticulata* (Jurine) in long term experiments. Lampert[7], using the same technique but with a 15-min exposure, reported a coefficient of selection greater than 1 for bacteria ingested by small daphnids in the presence of mixed food. I present here the results of a study of

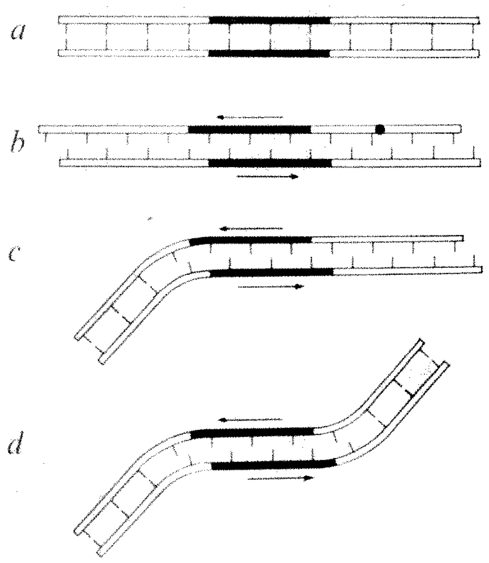

Fig. 2 Diagrams showing how a local active sliding (indicated by arrows) between two flexible filaments is converted into a bending. *a*, Before sliding. *b*, If there is no resistance to sliding between the filaments, active sliding does not lead to bending. This is presumably what happened to the trypsin-treated model of Summers and Gibbons[3]. *c*, If there is a region with resistance to sliding (stippled) on one side of the active region, a bend will be formed between these regions, compare Fig. 1*c*. *d*, If there is resistance to sliding on both sides of the active region, two bends will be formed in opposite directions, compare Fig. 1*b*.

one experiment, for example, bending of the proximal region required ATP at 2.0 nC while 0.2 nC was sufficient to straighten the same region. This indicates the possibility that once a bending wave is formed, it can be propagated in a concentration of ATP which is lower than is necessary to initiate a new wave. To test this, we placed the model in a solution containing 8 μM ATP. This concentration has been known to be lower than that required for the reactivation of bending, although enough to relax the "rigor waves"[8]. An iontophoretic application of ATP to the model in this solution induced a bending wave which was propagated down the flagellum. Propagated waves were also produced when the glass needle holding the sperm head was vibrated by a light tap at its handle. This also depended on the presence of a low concentration of ATP, since no such propagation was observed without ATP in the bathing solution. Our results indicate that a localised sliding between axonemal elements leads to bending, and also provide observations that may be important in understanding the mechanism of flagellar wave formation and propagation. The iontophoretic technique has proved useful for studying flagellar movement; it will be used to advantage in the studies of various aspects of cell motility at the cellular or organellar level.

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New approach to screening drugs for activity against African trypanosomes

THE African trypanosomes are unicellular eukaryotic parasites responsible for serious diseases in man (such as sleeping sickness) and domestic animals (such as nagana in cattle). The chemotherapy of these diseases is unsatisfactory[1]. Only a few drugs are available: these have serious side effects, drug resistance is a problem and "new drugs for clinical use are too expensive to develop and produce"[2] using classical empirical screening methods. We report here how knowledge of trypanosome metabolism has led to a new method for screening drugs against trypanosomes. This could provide a short-cut to new drugs or drug combinations.

The bloodstream forms of African trypanosomes are completely dependent on glycolysis for their energy supply, because the biogenesis of a mitochondrial respiratory chain is suppressed in the vertebrate host[3,4]. As Fig. 1 shows, aerobic glycolysis proceeds through the Embden–Meyerhof pathway similar to the glycolytic pathway of the host with the exception that pyruvate is excreted into the host's bloodstream as it cannot be metabolised further because long slender bloodstream trypanosomes lack both a functional Krebs cycle[4,5] and lactate dehydrogenase[6]. The NAD^+ reduced in the glyceraldehyde phosphate dehydrogenase step is reoxidised by a mitochondrial[7], CN^--insensitive L-glycerol-3-phosphate oxidase system[8] (boxed in Fig. 1), which is not present in the vertebrate host.

When the oxidase is completely inhibited by anaerobiosis or salicylhydroxamic acid (SHAM)[9], the bloodstream trypanosomes convert glucose into equimolar amounts of pyruvate and glycerol. According to Fig. 1, the net synthesis of ATP in this pathway should be zero. It has been demonstrated, however, that this is not the case and that high SHAM concentrations neither block trypanosome motility *in vitro*[9] nor affect the course of a fulminant *Trypanosoma brucei* infection in rats[10]. This has led to the conclusion that the scheme in Fig. 1 is oversimplified and that the bloodstream trypanosomes contain an, as yet unknown, extra

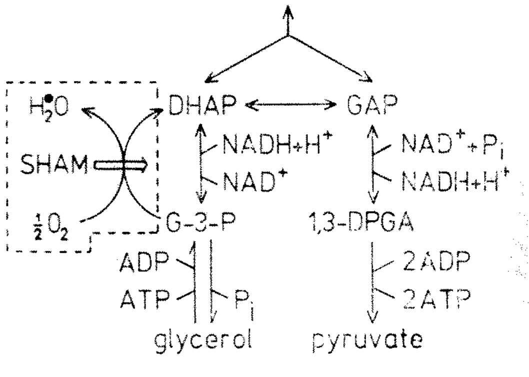

Fig. 1 Glycolysis in bloodstream trypanosomes. G-6-P, glucose-6-phosphate; FDP, fructose-1,6-diphosphate; GAP, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; G-3-P, L-glycerol-3p-phosphate; 1,3-DPGA, 1,3-diphosphateglycerate.

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Local reactivation of Triton-extracted flagella by iontophoretic application of ATP

IN spite of recent progress in ultrastructural and biochemical studies of cilia and flagella[1], the mechanism of their movement is still poorly understood. Much circumstantial evidence, however, supports the sliding microtubule model of ciliary and flagellar motility[2] which involves active sliding between axonemal microtubules to provide the motive force for bending. For example, ATP-dependent sliding of microtubules has been demonstrated in the Triton-extracted axoneme lightly digested by trypsin[3]. To produce a bend in the sliding microtubule model, there should be local differences in the amount of sliding along the axoneme. Morphological evidence indicates the presence of such differences in the bent region of cilia[4]. We now report experiments involving local, brief reactivation of Triton-extracted sea urchin spermatozoa by iontophoretic application of ATP. Our results support the idea of sliding within the axoneme as the basis of flagellar bending.

Spermatozoa from the sea urchin, *Anthocidaris crassispina*, were extracted as described before[5]. The extracted spermatozoa ('models') were placed in a 'reactivating solution without ATP' (for composition, see legend to Fig. 1) and a single model was held by attaching its head to a polylysine-coated glass needle[6] mounted on a micromanipulator. A glass capillary microelectrode, filled with a solution containing 10 mM ATP and with a resistance of about 500 MΩ, was used to activate the model locally by iontophoresis. The movement of the model was observed by an inverted microscope (Nikon MD) fitted with phase-contrast optics (objective: Nikon BM 40×), and was filmed on Kodak Double=X or Plus=X 16 mm film at 32–64 frames per s with a Bolex H16 ciné camera.

When the tip of the ATP electrode was placed close to the axoneme and ATP was applied as a negative pulse lasting 100–300 ms, the axoneme typically showed a localised, brief (less than 3 s) oscillation in the vicinity of the electrode. The frequency of observed oscillation was 2–7 Hz. The amount of ATP, expressed in nC, necessary to bring about the oscillation, varied with the specimen and also with the electrode used, but a few nC was usually sufficient. With this amount of ATP, it was possible to induce the response in any region of the axoneme, but the response was always localised and did not propagate beyond 5–9 μm of the electrode. This shows that any region along the length of the flagellum is capable of active bending[7], and also that a truly local application of ATP was achieved with our method. With less ATP, the duration of induced oscillation was shorter, and below a certain level a full cycle of movement was no longer obtained. Instead, the axoneme changed its form by a localised unidirectional movement of the region near the electrode (Fig. 1). By a few repetitive applications of the small amount of ATP to the same place, however, it was possible to induce one or more complete cycles. In other words, it was possible to break down a beat cycle of a segment of flagellum into a few steps.

The small amount of ATP applied to a more or less straight region of the axoneme typically elicited a local bending response (Fig. 1*a–c*). Usually, a pair of similar bends was formed in opposite directions (Fig. 1*a* and *b*) except in the distal region where only a single bend was formed (Fig. 1*c*). There was no fixed relation between the positioning of the electrode relative to the axoneme and the direction of bending, which seemed to depend on the orientation and other conditions of the axoneme. The above result is just what the sliding microtubule model predicts, if it is assumed that ATP causes a unidirectional sliding near the electrode and there is resistance to sliding outside the range of action of ATP. Diagrams illustrating this interpretation are shown in Fig. 2.

When the small amount of ATP was applied to the bent region, the bend moved some distance distally as the region directly under the electrode straightened (Fig. 1*d*). If the electrode was moved distally and ATP was applied to the newly formed bend, the bend was displaced further distally: this procedure could be repeated several times until the bend disappeared from the distal end of the flagellum. If there were two bends in the shape of an S, an application of ATP to the proximal bend had no visible effect on the distal bend: only the proximal bend moved distally (Fig. 1*e*). Similarly, ATP applied to the distal bend caused a distal displacement of this bend only, with no influence on the proximal bend.

The amount of ATP necessary to unbend the axoneme with consequent displacement of the bend tended to be lower than that necessary to bend the straight region. In

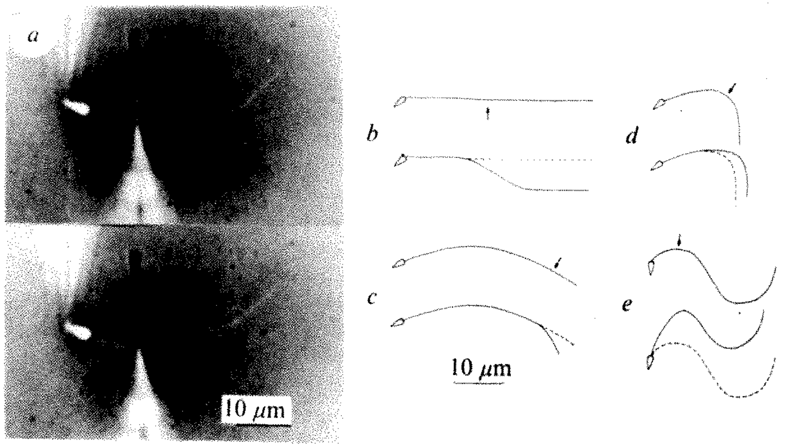

Fig. 1 Response of Triton-extracted flagella to local appliction of ATP. Spermatozoa of *Anthocidaris crassispina* were first suspended in 25–50 volumes of Ca-free artificial seawater buffered to *p*H 8.0 with Tris–HCl. Two drops of this suspension were added to 5.0 ml of extracting solution consisting of 0.15 M KCl, 4 mM $MgSO_4$, 0.5 mM EDTA, 0.5 mM mercaptoethanol, 0.04% (w/v) Triton X-100, 2 mM Tris–HCl, *p*H 8.0, at room temperature (25–28 °C), and were swirled gently for 30 s–1 min. The resultant suspension of extracted spermatozoa ('models') was transferred to 2 volumes of 'reactivating solution without ATP', which consisted of 0.15 M KCl, 2 mM $MgSO_4$, 0.5 mM EDTA, 5 mM dithiothreitol, 2% (w/v) polyethylene glycol and 20 mM Tris-HCl, *p*H 8.0, and was used immediately for local reactivation experiments. A single model was attached by its head to a polylysine-coated glass microneedle obtained by dipping the tip in 0.1% (w/v) polylysine, followed by a brief rinse in distilled water. At the end of local reactivation experiments, each model was exposed to bath application of 10–100 μM ATP, and models that failed to propagate normal waves were discarded. *a*, Prints from a 16-mm ciné film, showing a model both before and about 0.4 s after an iontophoretic application of ATP (0.13 nC). Note the position of the ATP-electrode and the induced local bending of the axoneme. *b–e*, Tracings from ciné films, each showing a model before (upper) and after (lower) application of ATP. Each arrow indicates the position of the ATP-electrode. The form of the axoneme before application of ATP is also shown superimposed on lower traces (dashed lines). The amount of ATP and the interval between the time of ATP pulse and the time of recording of the response were: *b*, 1.4 nC, 300 ms; *c*, 1.2 nC, 210 ms; *d*, 0.6 nC, 330 ms; and *e*, 0.8 nC, 350 ms, respectively.

up to about 3 μmol ml^{-1}, comparable with the level of histidine in histidinaemics. The offspring from treated mothers had no balance defects, deafness or vestibular damage (although litter size was reduced considerably on the 10% lysine or phenylalanine supplemented diets). The balance defect in offspring of histidinaemic mothers is therefore not the result of a simple increase in any amino acid to these concentrations during pregnancy although other, untested amino acids could be effective.

Table 4 Abnormalities of the inner ears of +/+ animals born to histidinaemic mothers after egg transfer

	Damage to inner ear			
	Left		Right	
Animal	Canals	Otoliths	Canals	Otoliths
1	Abnormal	Altered	—	—
2	Normal	Altered	—	—
3	Normal	Altered	Normal	Altered
4	Normal	Altered	Normal	Altered
5	Normal	Altered	Normal	Altered
6	Normal	Normal	Normal	Altered
7	Normal	Normal	Normal	Normal
8	Normal	Altered	Normal	Normal
9	Normal	Normal	Normal	Normal
10	Normal	Normal	—	—
11	Normal	Normal	Normal	Normal
12	Normal	Altered	Normal	Altered
13	Normal	Normal	Normal	Normal
14	Normal	Normal	Normal	Normal
15	Normal	Normal	Normal	Normal

Blastocysts were transferred 3.5 d *post coitum* into the uteri of recipient females 2.5 d *post coitum* by the technique of McLaren and Michie[13]. Animals 1–12 were C57BL +/+ eggs transferred into *his/his* hybrid females (50% Peru/50% C57BL). Animals 13–15 were Peru +/+ eggs transferred into *his/his* Peru females. All animals behaved normally and were not deaf. The ears were prepared and scored as in Table 1.

Our previous work[5] suggested that *his*/+ offspring from histidinaemic mothers were less severely affected than *his/his* offspring. Because the only offspring obtainable from histidinaemic mothers are of *his/his* or *his*/+ genotypes, we investigated this offspring-genotype effect further by +/+ egg transfers[12] into pregnant histidinaemic mothers. The transferred eggs had coat colour markers distinguishing them from the recipient's own eggs. No +/+ offspring with any visible balance defects or deafness were found. The ears of two groups of litters, however, showed subclinical damage (Table 4). This is significantly different from both +/+ and *his*/+ animals born of heterozygous mothers (which show no subclinical damage) but suggests that offspring genotype at the *his* locus in some way modifies the severity of the maternal effect.

Table 5 Effect of genetic background on the proportion of balance defective offspring produced by histidinaemic parents

			Affect on balance						Affect on hearing					
Genetic background	No. of litters	No. of animals	severe	No. of mild	normal	% Affected	χ^2	*P*	severe	No. of mild	normal	% Affected	χ^2	*P*
Peru	12	57	16	5	36	36.8	—	—	18	5	34	40.4	—	—
Hybrid Peru/C57BL	12	51	5	3	43	15.7	11.3	<0.02	8	1	42	17.6	12.4	<0.01

Some *his/his* mice from the Peru stock were crossed to C57BL and the offspring were intercrossed to produce *his/his* animals which were used as hybrid (50% Peru/50% C57BL) parents. The animals were scored for balance as in Table 1 and tested by χ^2 as in Table 3.

The original strain of Peru mice, in which the *his* allele occurred, had been selected for high penetrance of the balance-defective character without the knowledge of the presence of the allele and of the effect of maternal histidinaemia[5,13,14]. It is possible that this strong selection for several years may have accumulated modifying genes increasing the penetrance of the maternal effect on balance. These genes could operate through the maternal uterine environment or through the susceptibility of the offspring.

We tested this possibility by (1) putting the *his* allele into a different genetic background, and (2) relaxing selection for the balance defect. First, the Peru stock was crossed to C57BL mice, and offspring from histidinaemic mothers of this hybrid stock were examined (Table 5). There was a significant reduction in balance defective offspring (37% to 16%) from the hybrid stock although maternal histidine levels were the same in the hybrid and Peru strains (Table 1). Preliminary results of backcrosses of the *his* allele into C57BL suggest that the proportion of balance-defective offspring can be reduced still further. Similarly, a line in which selection was relaxed for 4 yr (about 12 generations) has reduced the proportion of balance defective offspring from more than 80% to below 10% although, again, the plasma histidine levels are the same in the relaxed and selected stocks. The nature of these modifying effects is being investigated. Preliminary results on reciprocal crosses between the 'high' and 'low' penetrance lines showed a low penetrance for both types of mother. This strongly indicates foetal susceptibility as the major component in penetrance difference and 'dominance' of 'low' over 'high' genes.

It is possible to draw some firm and some tentative conclusions from our observations. The developmental abnormality, whether subclinical or overt, is caused by abnormally high histidine or imidazole derivatives during the second week of pregnancy. These may be due to the genotype of the mother or to her diet. The teratogenic effect can be ameliorated (and possibly eliminated) by dietary management of genetically histidinaemic mothers during the critical period. The alternative explanation that the teratogenic effect is caused by the reduction of flux through histidase in histidinaemic mothers, is unlikely as heterozygotes have no reduction in flux (A. Whitehouse and G.B., in preparation) but can produce affected offspring when fed the 8% histidine supplemented diet.

The effect of the offspring genotype at the *his* locus on the penetrance and severity of the damage seems to be: *his/his*>*his*/+>+/+. This is, however, difficult to reconcile with the fact that histidase, which differs in adults in three genotypes, is not measurably different until about a day after birth. Furthermore, treatment of *his*/+ mothers during the third week of pregnancy was ineffective. A possible explanation involves the assumption that, contrary to other evidence, there are continuing metabolic effects on ear development after birth when the offspring's metabolism is significantly different in the three genotypes.

There is firm evidence that the genetic background is a contributing factor. This effect is, however, not due to any alteration in the histidine levels and it seems likely that it is offspring susceptibility rather than maternal uterine environment which is affected by modifying genes.

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Survival of dinoflagellate blooms in the western English Channel

DURING the summer of 1976, for the second successive year, blooms of the dinoflagellate *Gyrodinium aureolum* Hulburt have occurred at the western entrance to the English Channel. The influence of tidal mixing on the temperature structure of the water column and the availability of light and nutrients for phytoplankton growth in this region have already been described[1,2]. Here we report a frontal movement in response to the cycle of tidal mixing induced by the alternation of neap and spring tides, and suggest that plant populations both in surface water on the stratified side of the front and in the thermocline are dependent on the associated periodic release of nitrate and inorganic phosphate from the cold, nutrient-rich, bottom layer. We also describe the effects of phytoplankton on nutrient gradients across the thermocline.

To use ship time as efficiently as possible, both surface and subsurface measurements of temperature, salinity and chlorophyll *a* were recorded continuously while steaming[2,3]. Surface water was sampled through the ship's hull from a depth of 2 m, and sub- to surface water was pumped up (~ 100 l min^{-1}) from about 20 m through polyvinyl chloride tubing (4 cm internal diameter) attached to a V-fin depressor with a span of 1.3 m. Standard automated analytical techniques[4] were used to monitor silicate and nitrate in surface or subsurface water. Nitrate was chosen in preference to inorganic phosphate because, after the spring diatom outburst, the low (< 2) nitrate-N to phosphate-P ratios in surface waters suggest that nitrate is the limiting nutrient for the growth of dinoflagellates[5]. An example of records across a frontal boundary, where particularly high levels of chlorophyll a were encountered, is given in Fig. 1. Vertical profiles for the same water properties were obtained at a series of stations by lowering the salinity–temperature–depth measuring system and the inlet tube the pump into the water. Data on zooplankton distribution, based on samples taken from the outflow of the pump, will be reported elsewhere.

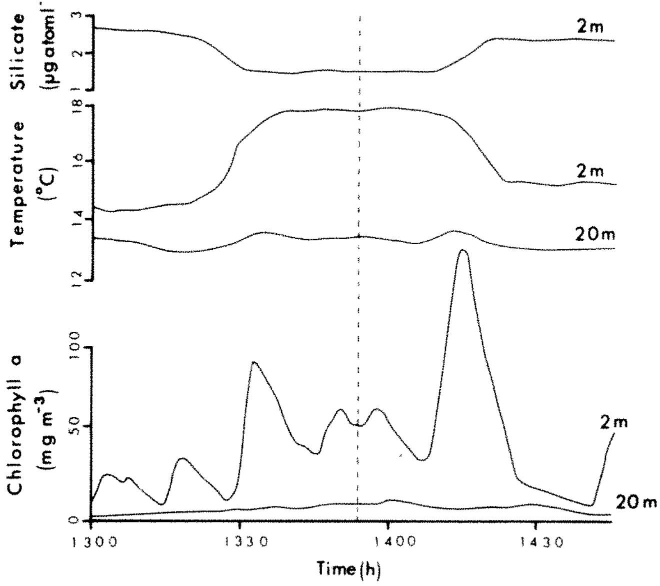

Fig. 1 Surface (2 m) and subsurface (20 m) records for chlorophyll *a*, temperature and silicate (surface only) across a frontal region (49 10′N, 4 48′W, August 3, 1976). At 1352 h the ship altered course to make a second crossing of the front (speed ~ 9 knots).

By comparing the records for temperature and chlorophyll *a* obtained just before (July 27–28, 1976) and just after (July 31–August 2, 1976) spring tides, the changes that occurred during the period of increased tidal mixing are clearly apparent (Fig. 2). In the former period the temperature front, as defined by the 15–16 °C contours, was close to the French coast. Then, as bottom turbulence increased at spring tides, the thermocline was eroded from below and a large area of cold well-mixed water (< 13 °C) was produced around the French coast. The frontal region between well-mixed water and stratified water had been displaced further towards the centre of the English Channel. At one place north-west of Ushant the vertical mixing was sufficiently severe to produce a reduction of nearly 6 °C in surface temperature.

The limits of frontal movement define an area where phytoplankton populations tend to be dispersed at spring tides but experience conditions suitable for growth as the water column stabilises at neap tides[1]. The most notable feature of the chlorophyll distribution maps, however, is the large area on the stratified side of the front where the concentration of chlorophyll *a*, which was already > 16 mg m^{-3}, appeared to increase during the period of strong tidal streaming. In the most dense patches of *G. aureolum* the water was chocolate brown in colour, and lines of white or yellowish brown detritus (Fig. 3) were again observed[1]. Associated with these scum lines, and also indicative of zones of water convergence, were reddish patches of *Noctiluca scintillans* (Macartney) Ehrenb., and large numbers of the copepod *Anomalocera patersoni* Templeton. Further to the west, where greater water depths and weaker tides make possible a deeper and more stable thermocline, and extending across the edge of the continental shelf, surface chlorophyll *a* values were generally less than 0.5 mg m^{-3}.

Vertical sections of water properties through the front show the position of the phytoplankton bloom in relation to the temperature structure and nutrient distribution (Fig. 4). While taking samples of phytoplankton from the thermocline, differences in the concentration gradients for nitrate and silicate were observed. In a typical vertical profile these are difficult to demonstrate because of the time lag of the analyser system and because most of the change between surface and bottom mixed layers occurs in a few metres within the thermocline. But by taking advantage of the calm conditions and leaving the hose inlet and temperature probe at a fixed depth corresponding to the mean depth of the chlorophyll-rich layer, it was possible to measure levels of the two nutrients at various positions across the temperature gradient as the passage of internal waves (period 5–10 min) caused slow up and down movement of the thermocline[6]. Figure 5 shows that while there is approximately a one to one correspondence between the temperature and silicate traces, the nitrate values increase significantly only

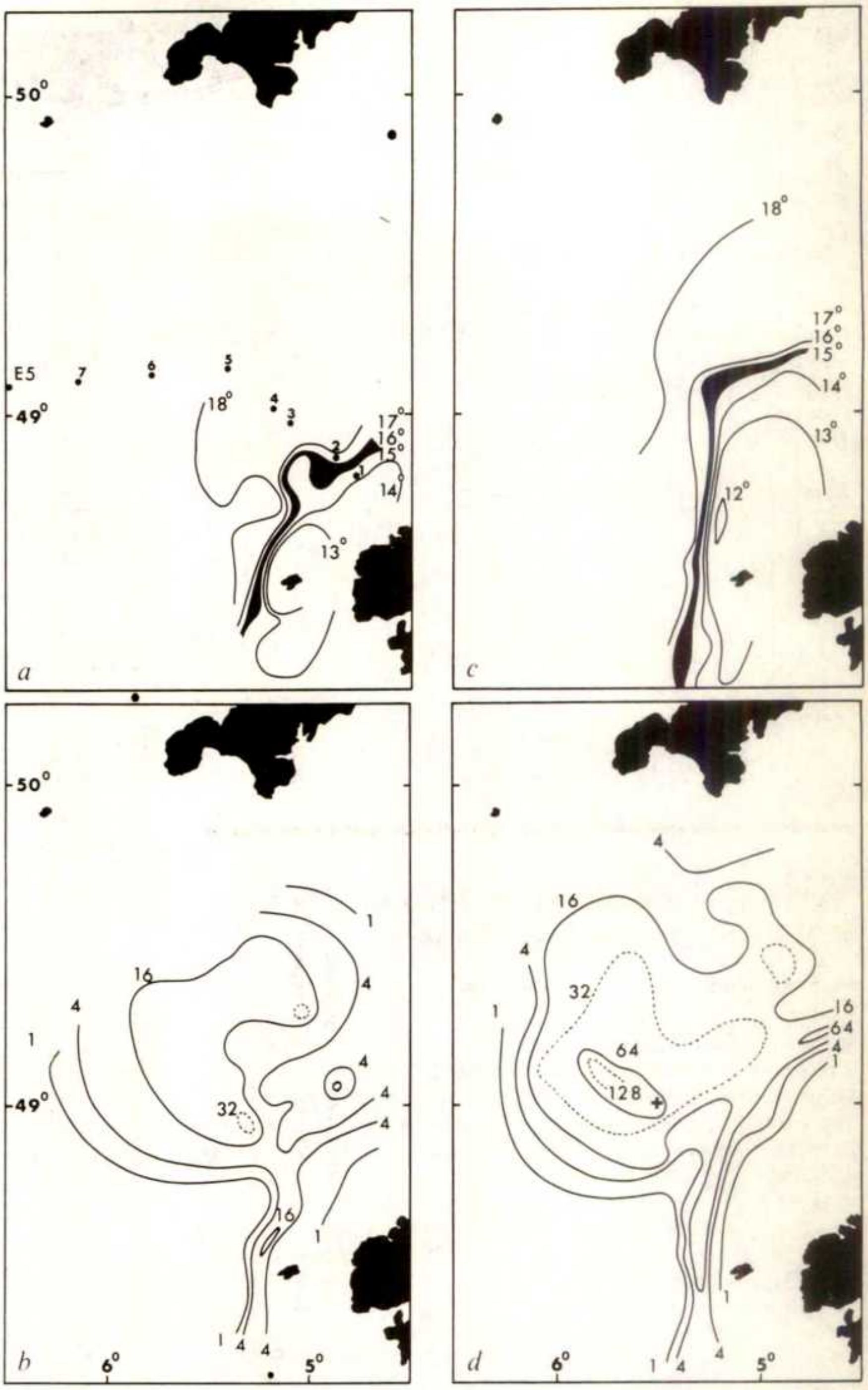

Fig. 2 *a*, Surface temperature (°C); *b*, surface chlorophyll *a* (mg m^{-3}) distributions for July 27–28, 1976. *c*, Surface temperature (°C); *d*, surface chlorophyll *a* (mg m^{-3}) for July 31–August 2, 1976 (+ marks the position at which Fig. 3 was taken).

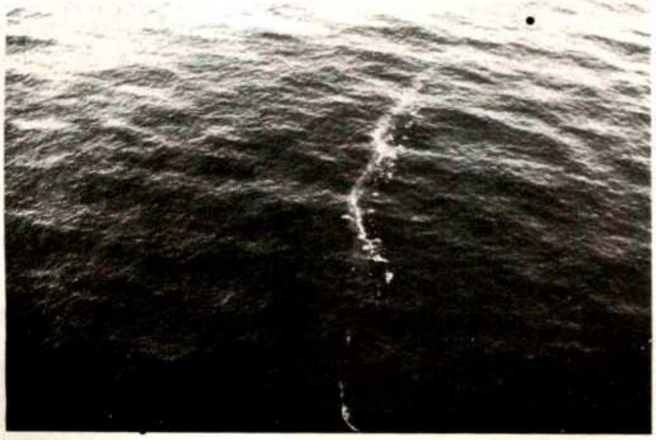

Fig. 3 Surface scum at 49°00'N, 5°30'W Fig. 2*d*), 1150 h GMT, August 9, 1976, from 100 m.

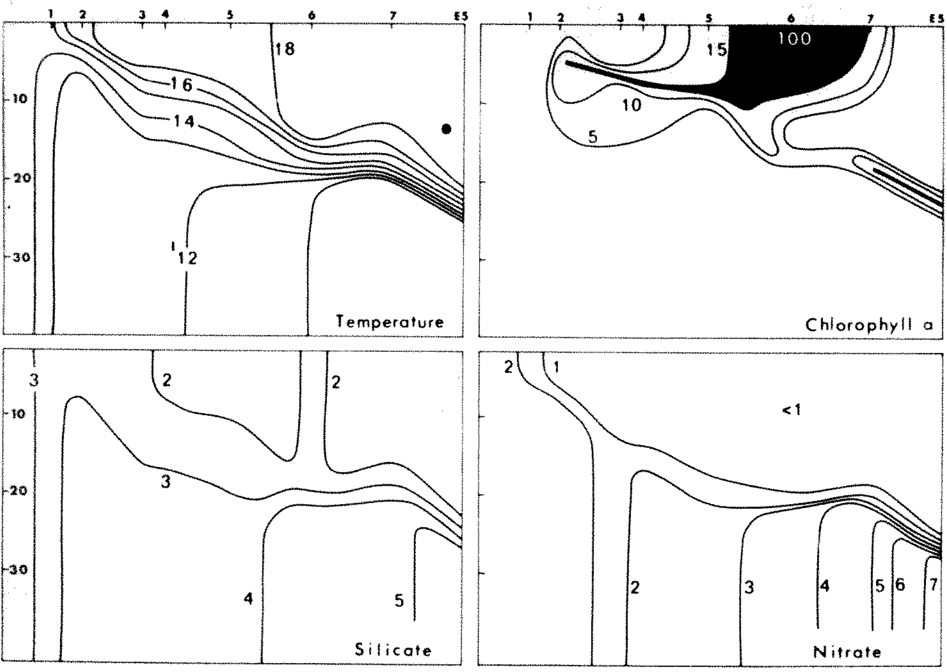

Fig. 4 Vertical sections from temperature (°C), chlorophyll *a* (mg m^{-3}), and silicate and nitrate (μg atom l^{-1}) from mixed (station 1) to well stratified water (station E5). The station positions are shown in Fig. 2*a*. Extreme values for inorganic phosphate, the distribution of which was similar to nitrate, were 0.13 and 0.71 μg atom l^{-1} for surface and bottom water at station E5.

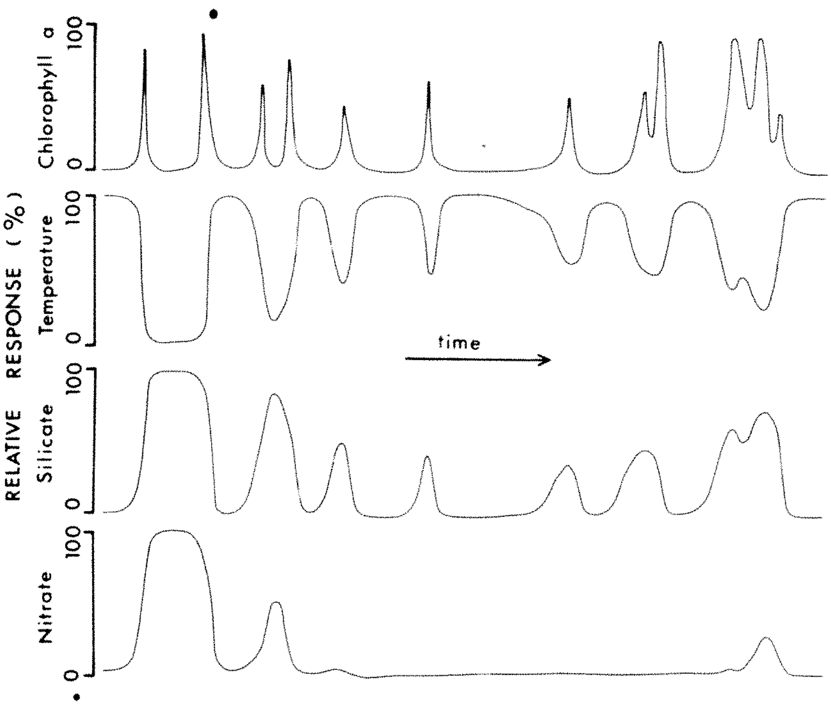

Fig. 5 Records from station E5 (1450–1610 h GMT, August 10, 1976) for nitrate, silicate, temperature and chlorophyll *a* at the mean depth of the thermocline (25 m). Each is presented on a scale of percentage change between values for the surface and bottom mixed layers (Fig. 4).

near the base of the thermocline and below the chlorophyll peak. Analysis[7] of discrete samples indicates that the vertical distribution of inorganic phosphate is similar to that of nitrate.

Dinoflagellates, unlike diatoms, do not have a high requirement for silicate and this is reflected in the relatively high surface values for silicate (Fig. 4)[8]. But the survival of dense populations of these organisms, in both the thermocline and frontal surface water, must depend on an injection of nitrate and inorganic phosphate and this, we suggest, takes place at the base of the thermocline during spring tides at fortnightly intervals. Where the thermocline is relatively shallow, the increased bottom mixing at this time will also affect surface water. Thus the eastern boundary of the more persistent part of the dinoflagellate bloom is marked by the position of the front at spring tides, whereas the western limit is controlled by a deeper and more stable thermocline through which there is little or no exchange of water properties between the surface and bottom mixed layers. Any upward movement of the plant cells during spring tides will minimise the risk of thermocline populations being mixed downwards into the poor light regime of the bottom mixed layer, as well as help to explain the very high surface chlorophyll values which are more than ten times those found in typical spring outburst conditions[2]. We predict the occurrence of similar phytoplankton blooms and gradients in nutrient concentrations on other parts of the Shelf region of the British Isles where comparable hydrographic conditions exist; for example in the North Sea near the Farne Islands, to the east of Orkney and Shetland, and to the west of the Outer Hebrides[9].

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bounding the external acoustic meatus and ensheathing a bony styloid process which is still in place. Portions of the base of the cranium are available, including the lateral occipital with occipital condyle and the posterior part of the basi-occipital; portion of the floor and anterior wall of the middle cranial fossa, including the root of the lesser wing of the sphenoid bone. One lateral pterygoid lamina has been identified.

Of the face, there are present both nasal bones, complete from nasion to rhinion, supported by the left and right frontal processes of the maxilla. The pyriform aperture is virtually complete, including part of the floor of the nasal cavity. Most parts of the two maxillary bones are available, including the subnasal clivus, the palate and the alveolar processes. We have a large portion of the palate; the two maxillae have disarticulated neatly in the middle line. All of the front tooth sockets are present, though none of the front teeth has yet been found. On the right, all five cheek-teeth from P^3–M^3 are available, with superbly preserved crowns and intact root systems. On the left, four of the five cheek-teeth are present; only the M^1 is lacking.

The mandible is thus far represented by only a good piece of the right ramus and possibly one or two additional fragments.

Morphology of calvaria

Salient features of the cranial and dental morphology are as follows. The skull as a whole is very small, though the curvature of the vault bones in the temporal fossa is full and rounded, quite unlike the marked constriction of this region found in the australopithecines, for example, Sts 5.

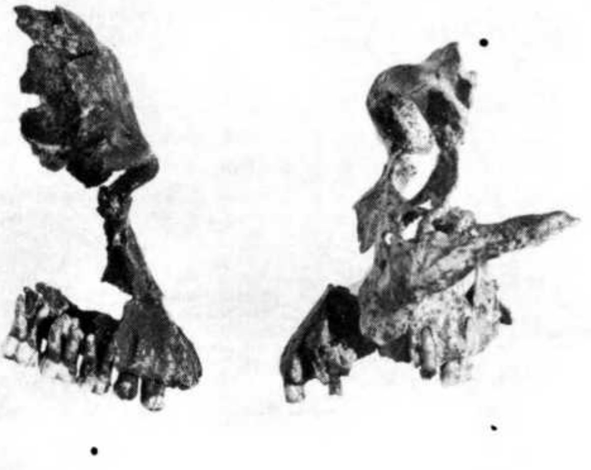
It is impossible at this stage to give an estimate of the cranial capacity, but we are confident that enough of the vault is preserved to make a reasonable estimate possible later. Two indications suggest a capacity that is moderately large for so small an individual: first, the well-filled vault in the temporal fossa and, secondly, the disposition of the temporal lines. These lines are modestly developed and they are well separated from the midline, both anteriorly on the frontal bone and posteriorly on the left parietal bone. The morphology of these temporal lines strongly suggests the pattern in *H. habilis* of East Africa.

The brow is very thin and moderately protuberant; in its delicacy and slight vertical thickness, it is reminiscent of Olduvai hominids 16 and 24, two of the specimens that have been referred to *H. habilis*.

The nose

The nasal bones are set at 180° to each other, except superiorly, where they have a slight linear elevation in the midline just below nasion. The well-preserved floor of the nose, anterior nasal spine, nasal sill and subnasal maxilla are virtually identical to the maxilla from Swartkrans (SK 80) that Robinson[8] recognised as 'Telanthropus III'. It was SK 80 that R. J. Clarke later found to join perfectly with cranium SK 847 and which he and Howell[9] described as belonging to a species of *Homo*. The criteria that Robinson[8] enunciated to distinguish between this area in *Homo*, on the one hand, and *Australopithecus* (both *A. africanus* and *A. robustus*), on the other hand, are still valid and they clearly distinguish the new Stw 53 maxilla from that of *A. africanus* and align it with *Homo*.

Fig. 1 Maxilla of the new Sterkfontein skull, Stw 53, compared with SK 80, a Swartkrans maxilla of *Homo* species, originally called 'Telanthropus III'.



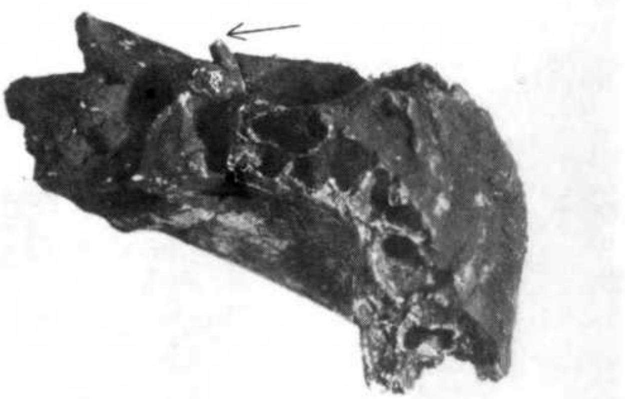


Fig. 2 Base of temporal bone of Stw 53 showing the ossified styloid process (arrowed).

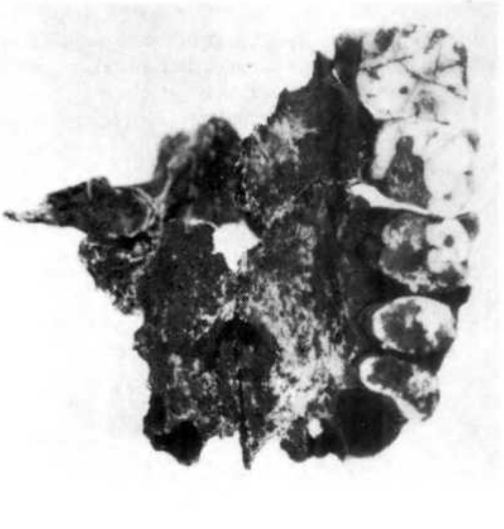
Cranial base

On the base of the cranium, the presence of an ossified styloid process is a feature that distinguishes Stw 53 from all previously described specimens of *Australopithecus* (Fig. 2). The lateral pterygoid plate, as R. J. Clarke pointed out to us, has a nearly vertical, notched posterior margin, like that of *Homo* and unlike the full, posteriorly-expanded, oblique posterior margin, in both robust and gracile australopithecines.

The teeth

The teeth are heavily worn, especially P^3, P^4, M^1 and M^2, while there is moderate attrition even on the M^3 (Fig. 3).

Fig. 3 Palatal view of right maxilla with all five cheek teeth from P^3 to M^3 in position five.



A most striking feature of the teeth is the number and complexity of the roots. Both the P^3s and P^4s have three roots each, two buccal and one lingual. The position on P^3 is encountered in a few of the Sterkfontein *A. africanus* teeth: according to Sperber[10], of 7 Sterkfontein P^3s, 1 has a single root, 3 possess two roots and 2 have three roots. Thus, in comparison with the Member 4 sample from the same site, the roots of the Stw 53 premolars are unremarkable. The position is, however, different with the P^4. All 8 *A. africanus* P^4s, the roots of which could be observed, display two roots[10]. In this respect, the P^4 of the new Member 5 skull distinguishes itself from all specimens thus far known from Member 4 (Fig. 4).

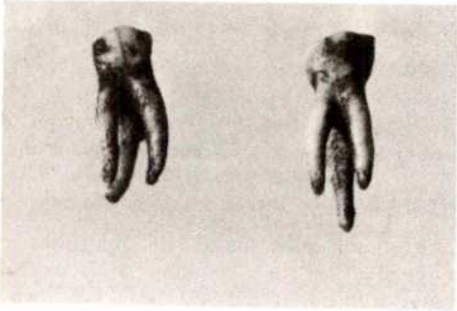


Fig. 4 Upper third and fourth premolars (isolated) to show the three roots of each.

It is interesting to note that in the Swartkrans maxilla (SK 80), which the Stw 53 so strongly resembles, Robinson states, " . . . it is quite clear from the sockets on the right side that P^3 had three separate roots, the mesio-buccal one being closely approximated to the canine root"[8]. Unfortunately, only a part of the root and crown of "what is probably the right P^4" is present and he did not refer to the root pattern reflected in the sockets for the P^4 roots.

Three-rooted premolars are common in the Swartkrans fossils attributed to *A. robustus*. Thus, 8 out of 14 P^3s and all but one of the P^4s, from Swartkrans have three roots[10].

There is a highly distinctive and florid development of the root system in the three molars. Each is furnished with two buccal roots and a sturdy lingual root, but each of these three roots is itself manifestly double and, at the tips, partially bifid. The duality of each of these three roots is more striking in M^2 than in M^1 and the degree of separation of each pair of two components is more marked. This is especially true of the mesio-buccal root, whose lingual component is so sundered from the buccal component as to place it virtually in the bucco–lingual midline of the tooth; its appearance is abnormal and this lingual rootlet curves strongly bucco–distad. The graded series apparent from M^1 to M^2 is continued into M^3, where the process of root doubling of the mesio–buccal root reaches a climax with the marked distal recurvation of its lingual moiety (Fig. 5). In contrast M^3 shows less duality of the disto–buccal root and of the lingual root. This pattern is sharply distinct from those of the australopithecines: in the latter group, scarcely a single one of the upper molars shows even a trace of grooves on the roots, let alone a frank doubling of each!

The mandible

The mandibular ramus is slender and gracile. The sculpturing on its medial face is clear cut and there is an acute angle between the endocoronoid and endocondyloid crests. The mandibular fossa is moderately developed as a flange in front of the endocoronoid ridge. The mandibular notch is somewhat excavated and it is fronted by an acuminate coronoid process. There is a distinct lingula. The features bear comparison with the corresponding part of Olduvai hominid 13 ('Cinderella'); but in the latter, the sculpturing is accentuated and there is not so distinct a lingula. The part as a whole bespeaks a high, narrow, modern-looking ramus of the lower jaw.

All these features add up to a skull that seems to have a preponderance of individual features aligning it with early *Homo* species. It clearly has very little in common with *H. erectus* and would seem to be far too small-brained, thin-skulled and mildly crested to be accommodated in that taxon. On the other hand, the total picture indicates a strong affinity with the small-skulled, moderately-brained and structurally distinctive *H. habilis*. Stw 53 differs from *A. africanus* in much the same way as does the roughly contemporary, pygmoid, brainy *H. habilis* of East Africa, now known from Olduvai (1.8 Myr), Omo (1.85 Myr)[11] and East of Lake Turkana (?2.0–1.8 Myr)[12].

Conclusions

In this new discovery, we believe we have for the first time, convincing evidence that the Sterkfontein tool-maker belonged to the genus *Homo* and either to *H. habilis* or to a form closely related (*H. aff. habilis*). Furthermore, the positions of *A. africanus* in Member 4 and of *Homo* and stone tools in Member 5 strengthen the long-established hypothesis that *A. africanus* was earlier in time than early *Homo* (such as *H. habilis*).

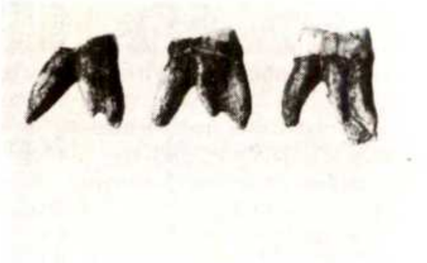


Fig. 5 Mesial views of M^1, M^2, M^3 to show the increasing degree of separation, from M^1 to M^3, of the mesio-buccal root into two rootlets. The lingual component of the mesio-buccal root becomes progressively closer to the bucco-lingual midline of the tooth and is strongly recurved buccally and distally.

This view has gained important support from the newest evidence from East Africa[11,12]. Moreover, the relative ages of *A. africanus* and *H. aff. habilis*, as revealed at Sterkfontein, supplemented by the evidence from East Africa are in keeping with the widely-held and strongly supported view that *A. africanus* is still the most likely claimant to have been ancestral to *H. habilis* and to later species of man.

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and decreases with the square of the deviation from this optimum amount, then, if mating is random and if recombination is equally likely to increase or decrease the genome length, Crow and Kimura[40] show that the chromosome length will be normally distributed about $N = N_0$. The gambler's ruin model should thus only be considered as an idealised model which gives another and independent estimate of the gene fixation time.

Multiple mispairing

To estimate the fixation time with multiple mispairing we shall use the diffusion equation method of population genetics[40]. If x is the frequency of occurrence of a particular repeat, say the ith, we need to compute the mean $M_{\Delta x}$ and variance $V_{\Delta x}$ of the frequency change per unit time. We shall continue to assume that there is no selection so that $p = 1/2$. To maintain $N(t) = N_0$, however, we shall follow Ohta[41] and assume that unequal crossovers occur in cycles composed of a duplication of m repeats followed by a deletion of m repeats, where the amount of mispairing per cycle, m, is a random variable. If the repeats of type i are distributed randomly among the N_0 repeats of the multigene family, then the probability of increasing the number of type i repeats by $\xi_1 N_0$ during a duplication event in which the mispairing is by m is given by the binomial distribution

$$P_{x\to x+\xi_1} = \binom{m}{\xi_1 N_0} x^{\xi_1 N_0}(1-x)^{m-\xi_1 N_0},\quad 1 \leqslant \xi_1 \leqslant m \qquad (18)$$

Analogously, the probability of then decreasing the number of type i repeats by $\xi_2 N_0$, given a mispairing by m, is

$$P_{x+\xi_1\to x+\xi_1-\xi_2} = \binom{m}{\xi_2 N_0} x^{\xi_2 N_0}(1-x)^{m-\xi_2 N_0},$$
$$0 \le \xi_2 \le m \qquad (19)$$

where we have neglected the small changes in the gene frequency and repeat length caused by the preceeding duplication event. Thus, for a series of cycles with random mispairings m occurring with frequency $f(m)$, and ξ_1 and ξ_2 taking on all possible values for a given m,

$$V_{\Delta x} = \sum_{m=1}^{N_0} f(m) \sum_{\xi_1=0}^{m} \sum_{\xi_2=0}^{m} (\xi_1-\xi_2)^2 P_{x\to x+\xi_1} \times P_{x+\xi_1\to x+\xi_1-\xi_2} \qquad (20)$$

$$= \frac{2\bar{m}x(1-x)}{N_0^2} \qquad (21)$$

where

$$\bar{m} = \sum_{m=1}^{N_0} m f(m)$$

Similarly, $M_{\Delta x} = 0$.

Using the results of Kimura and Ohta[42] we then find that the mean fixation time measured in crossovers is

$$t_1 = \frac{-2N_0^2}{\bar{m}}(N_0-1)\ln(1-1/N_0) \qquad (22)$$

For unit mispairing the same results obtain with $\bar{m}$ replaced by one[41]. Thus the effect of multiple random mispairings is to decrease the fixation time by the mean mispairing $\bar{m}$.

Ohta[41], using a similar diffusion equation analysis for the case of unit pairing unequal crossovers, computed $V_{\Delta x}$ and t_1 and obtained equations (21) and (22) with $\bar{m} = 1$. If we compare the diffusion equation results with the fixation time estimate derived by the birth–death process we find

$$t_1(\bar{m} = 1) = -N_0\ln(1-1/N_0)T_{\text{bd}} \sim (1 + \frac{1}{2N_0} + \frac{1}{3N_0^2} + \ldots)T_{\text{bd}} \qquad (23)$$

where T_{bd} is given by equation (11). As N_0 becomes large the two estimates converge to the same result. For small values of N_0, T_{bd} is probably a more accurate estimate since we do not need to assume that the repeat frequencies are continuous variables nor do we need to assume that duplication and deletion events strictly alternate (although we do assume that the average number of duplications and deletions are equal).

In Smith's[27] Monte-Carlo simulations $N(t)$ varied between 0.9 N_0 and 1.1 N_0. For $N_0 = 500$, mispairings between 1 and 50 occurred. If we assume that these occurred uniformly then $\bar{m} \approx 25$ and $t_1 \approx 2\times10^4$, which is in agreement with the Monte-Carlo results.

Future problems

We have constructed four mathematical models of unequal crossing over between sister chromatids. For the case in which there are equal probabilities of the offspring retaining the expanded or contracted chromosome (that is $p = 1/2$), we have given explicit solutions. The solutions have been interpreted to show that at long times one gene in a multigene family becomes fixed while the other genes are lost. Hence, as Smith[27] has pointed out, if the average time for a gene to acquire a mutation (the gene mutation time) is long compared with the time for gene fixation a very homogeneous multigene family would result which would seem to show coincidental evolution. On the other hand, if the gene mutation time was short compared with the gene fixation time a heterogeneous multigene family would be formed.

One might expect that selection would influence the relative probabilities of retaining the expanded or contracted chromosome. In *D. melanogaster* Tartof[13] has found that even though expansion and contraction are concurrent events, offspring containing an expanded amount of rDNA are more prevalent than offspring containing a decreased rDNA content. It would be interesting to solve equations (1)–(3) or the related multidimensional random walk problem when $p \neq 1/2$ and $N(t) \neq$ constant. Mathematically, one can view this unit mispairing problem as an urn problem in which N_0 balls each of a different colour are placed in a container at $t = 0$. A ball is chosen at random and replaced by two balls of the same colour as the chosen ball with probability p, and not replaced with probability $1-p$. We present this problem as a challenge to mathematicians.

We have shown that, using a diffusion model, the time to gene fixation can be estimated for unequal crossing over in which the mispairing is a random variable. But we had to impose the rather stringent and biologically unreasonable assumption of the crossovers occurring in cycles of duplication and deletion events of identical length. Further, we assumed that the repeats were randomly arranged along the chromosome since correlations in the positions of initially adjoining genes will influence the probabilities of genes being duplicated or eliminated by unequal crossovers in which mispairing occurs by two or more repeats. To assess these correlations in analytical form and to relax the constraint that $N(t) = N_0$ seems to us to be an extremely challenging and difficult problem which we hope will attract some mathematical attention.

A realistic, but difficult mathematical problem is to enlarge the basic model so as to allow crossing over between homologous chromosomes in a diploid organism. At least in the case of *D. melanogaster* this does not seem to be necessary, for Tartof[13] has found that unequal crossing over occurs predominately between chromatids on the same chromosome. Whether such restrictions also occur in other species where unequal crossing over has been adopted as an evolutionary mechanism remains to be seen.

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A fossil skull probably of the genus *Homo* from Sterkfontein, Transvaal

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A skull with numerous Homo *features was discovered at Sterkfontein, near Krugersdorp, Transvaal, in August 1976. It stems from member 5 of the Sterkfontein Formation, with stone tools and with fauna pointing to an age of 2.0–1.5 Myr. The underlying member 4 contains* Australopithecus africanus, *no stone tools, and fauna dated 3.0–2.5 Myr. The new find supports the view that the Sterkfontein toolmaker was not the earlier* A. africanus, *but a later hominid related to* Homo habilis.

By a remarkable double coincidence, the first pieces of the new skull, Stw 53, were found on August 9, 1976, 40 years to the day after Robert Broom's first visit to Sterkfontein, while the last part came to light on August 17, the 40th anniversary of Broom's first discovery of a hominid cranium at Sterkfontein. Although most of the fragments were found in a decalcified pocket of cave earth, one large part of the calvaria was still present in the calcified wall of the pocket, thus establishing indisputably the provenance of the specimen.

THE Sterkfontein cave deposit near Krugersdorp, Transvaal, has previously yielded a large number of fossil hominid bones that form the major part of the hypodigm of the species, *Australopithecus africanus*[1]. Recently we extended our operations to a previously overburdened area south of the West Pit (formerly called the Extension Site). Here, between August 9 and 17, 1976 we brought to light a new fossil skull which, we believe, provides the first good evidence that the stone tools in the upper part of the Sterkfontein cave deposit were made by a hominid very similar to the early species of man originally described from Olduvai Gorge and designated *Homo habilis*[2].

The discovery was made 3 m south of the breccia from which, in 1956–1958, Brain and Robinson[3] had recovered the first stone tools from Sterkfontein. The breccia exposed in this part of the cave comprises Member 5 of the Sterkfontein Formation, according to Partridge. Member 5 contains a mammalian fauna that is much younger than that of the subjacent Member 4: according to Vrba's[4,5] study of bovids in the two Members and other evidence, there may well be a lapse of ~0.5–1.0 Myr between the two Members. The long gap in the depositional sequence is the result of a marked unconformity between Members 4 and 5.

Apart from strong differences in bovid fauna, Members 4 and 5 differ in other respects: 4 contains no stone implements, while 5 contains an abundance of them. All of the hominid fossils unequivocally assigned to *A. africanus* emanate from 4, while until the new find was made, Member 5 had yielded only a handful of hominid teeth and jaw fragments. So unsatisfactory were these Member 5 remains that the identity of the hominid represented was uncertain. Robinson[6] considered them to belong to *A. africanus*, like those from the deeper part of the cave, while Tobias[7] regarded them as belonging to the genus *Homo* and probably to the species *H. habilis*. The new discovery provides the first unequivocal evidence of the nature of the Member 5 hominid and, probably, of the Sterkfontein stone tool-maker.

The new skull Stw 53

It is clear that we have a large part of the complete skull. We have the right brow, frontal bone and partially exposed frontal sinuses, several parietal fragments, including a posterior part of the left parietal bone articulating across the lambdoid suture with part of the occipital. The occipital includes a portion of the planum occipitale as well as of the planum nuchale, with a small, sharp nuchal crest on the medial, but not the lateral part of the bone. On the side of the cranium, we have a temporal, with well-preserved mastoid process, petrous pyramid and part of the tympanic

letters to nature

On the optical observation of galaxies with large redshifts

IT IS well known that the energy distributions of spiral galaxies rise sharply in the ultraviolet. Recent observations of six Sb and Sc galaxies[1] made with the TD1 satellite place this steep rise at considerably longer wavelengths than the earlier observations made with the OAO-2 satellite[2,3] If the TD1 results are correct, the large ultraviolet flux, which is now considerably closer to the U band, suggests the possibility of detecting more distant galaxies with deep ultraviolet plates than the usual deep B, V or R plates, since this region of high flux will be redshifted into the U band.

The problem of whether distant galaxies fall within the limiting apparent magnitude of a plate can be examined from the published energy distributions. Figure 1 shows the absolute energy distribution adopted for an Sb/Sc galaxy. Below 2740 Å the fluxes represent the average of the six galaxies measured by the TD1 satellite, the various intrinsic luminosities being allowed for by using published V magnitudes[4] and normalising to a constant V magnitude. Above 2700 Å the relative fluxes taken from Pence[3] were made absolute by using the flux for M31 (ref. 5) scaled to the chosen V magnitude, Pence's results below 3500 Å are, however, an extrapolation. It is encouraging to note the continuity between the two portions of the energy distribution, considering the large errors which may be involved.

Now the apparent magnitude, m, of a galaxy with absolute magnitude M is simply

$$m = M + 25 + 5\mathrm{lo}_{10}(d_L) + K_1(z) + K_2(z) \qquad (1)$$

where d_L is the luminosity distance in Mpc given by[6]

$$d_L = \frac{c}{H_0 q_0^2}(q_0 z + (q_0 - 1)\{(2q_0 z + 1)^{1/2} - 1\}) \qquad (2)$$

and the K-corrections are

$$K_1(z) = 2.5\log_{10}\left[\int_0^\infty F(\lambda)S(\lambda)\mathrm{d}\lambda \Big/ \int_0^\infty F\{\lambda/(1+z)\}S(\lambda)\mathrm{d}\lambda\right] \qquad (3)$$

$$K_2(z) = 2.5\log_{10}(1 + z) \qquad (4)$$

where $F(\lambda)$ is the radiation flux at wavelength λ and $S(\lambda)$ is the detector response. Following Oke and Sandage[7] we have separated the effect of the redshift on the bandwidth, that is, the K_2 term, from the effect of simply translating to the redshifted wavelength, that is, the K_1 term. Our point is that for the U band, K_1 eventually starts decreasing with redshift, whereas K_2 increases monotonically. The essence of the present problem is the competition between these two terms.

For a deep plate, the detector response will depend on the film, its filter and the sky transmission. As the flux has only been measured for a few wavelengths, we have made the approximation

$$K_1(z) = 2.5\log_{10}\left[F(\lambda_e)\Big/F\left\{\lambda_e/(1+z)\right\}\right] \qquad (5)$$

with effective wavelengths λ_e of 0.33 μm for the U band and 0.44 μm for the B band. Using the curve in Fig. 1 together with equations (1), (2), (4) and (5), we have calculated the apparent magnitude for the Sb/Sc galaxy at various redshifts. This was done for $q_0 = 0.2$ and for an absolute magnitude corresponding to the turn in the differential luminosity function. This occurs at $M_B^* = -21$ mag 1 and $M_U^* = -21$ mag 2 for $H_0 = 50$ km s^{-1} Mpc^{-1} and U − B = −0 mag 1 (ref 3). The results are shown in Fig. 2. A calculation with $q_0 = 0.02$ leads to little difference for redshifts below 1.0.

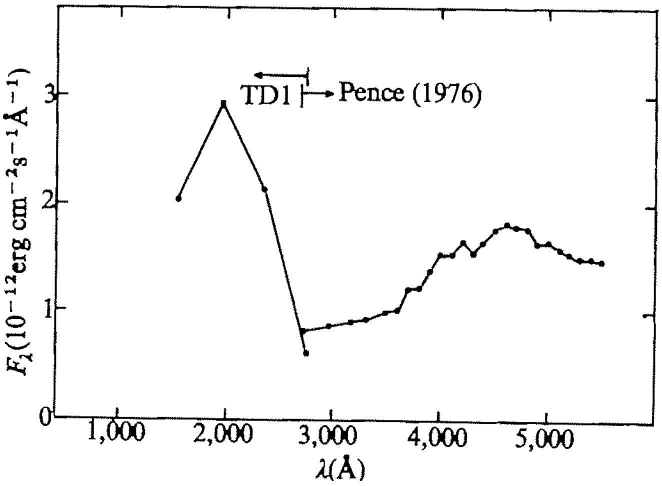
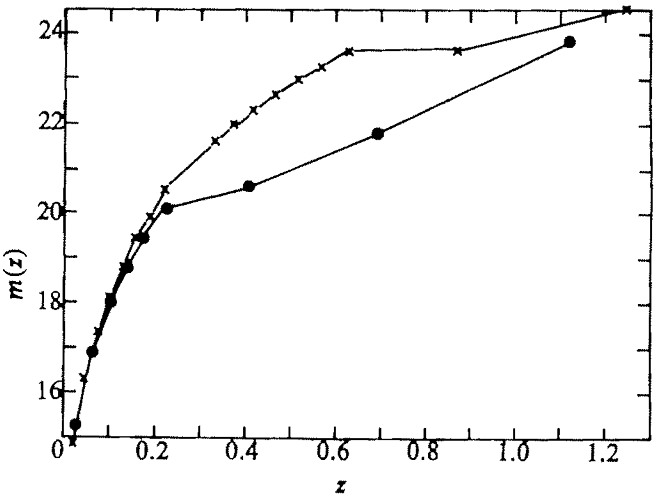


Fig. 1 Observed absolute spectral energy distribution for an Sb/Sc galaxy, normalised to $V = 8$ mag 49 (NGC 4258)

The distribution of visible galaxies with redshift on a U plate depends mainly on its limiting apparent magnitude We have been unable to calculate this directly; instead we have arrived at an approximate value by comparing each factor involved with its appropriate counterpart when taking a deep B plate The sensitivity of a B plate (nitrogen-soaked Kodak IIIa-J plus Schott GG395 filter) is close to that of a U plate (nitrogen-soaked Kodak

Fig. 2 Apparent U and B magnitudes at various redshifts ×, $m_B(Z)$; ●, $m_u(Z)$.



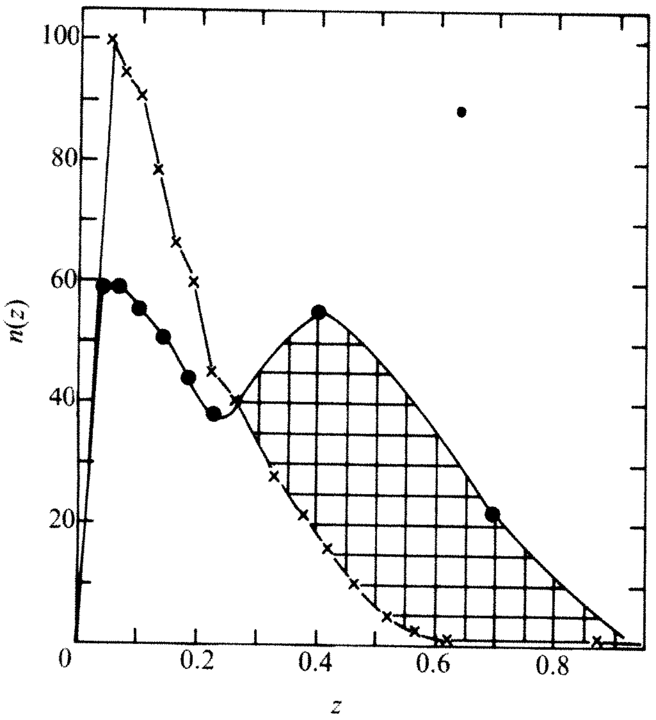


Fig. 3 Relative numbers of Sb and Sc galaxies visible on deep B and U plates at various redshifts. The hatched area represents the excess observed when blinking from the B plate to the U plate. ●, U plate; ×, B plate.

Ha-O plus Schott UG1 filter)[8,9]. The major difference with deep U plates lies in the sky brightness. For the two wavelengths considered, Simkin[10] quotes U–B = −0mag 7. It has already been demonstrated that images as faint as B = 23mag 0 have been recorded on IIIa-J photographs taken with the UK 48-in Schmidt. Hence we have considered here a limiting magnitude of U = 22mag 3 as fairly realistic.

We can show that there is an excess of distant images on the U plate, as compared with those on a B plate. These could be readily identified by comparing the images on the two plates, for example via the COSMOS machine[11]. In order to estimate how many galaxies would be on the U plate but not on the B plate, we have used Christensen's[12] absolute luminosity function. The loss of galaxies caused by atmospheric 'seeing' has been ignored. The effect of evolution has also been neglected, but it can be readily seen that the predicted time variation of the energy distributions[13] will make the detection of distant galaxies even more favourable, mainly because distant, and thus younger, galaxies will be more luminous. The frequency of galaxies at various redshifts for plate limits B = 23mag0 and U = 22mag3 is plotted in Fig. 3. The excess shaded region on the graph corresponds to about 700 Sb and Sc galaxies for an area of 1 square degree, that is, about 15% of the total number of all types on 1 square degree of the B plate. The interesting feature is that most of these galaxies lie in the redshift range of 0.3–0.8.

As in the identification of QSOs, blinking between U and B plates can produce a host of other interlopers[14], though the images sought here are close to the plate limit (Fig. 2); further comparisons with deep red plates might eliminate some of these. The fainter QSOs might be expected to have very large redshifts and neutral colours[15] and hence appear on all three plates.

The suggestion depends on the approximate validity of the TD1 results. With respect to the discrepancy between the TD1 and OAO-2 results, we note that there is a similar discrepancy between the OAO-2 and the recent Skylab ultraviolet observations[16]. There is evidently an urgent need for further accurate extragalactic observations in the ultraviolet. Deep U plates have been taken recently with the UK 48-inch Schmidt telescope and we intend to pursue the matter discussed in this note further using the COSMOS machine.

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The polarisation of nova Vulpeculae

MEASUREMENTS of the polarisation of nova Vulpeculae 1976 are reported. The linear polarisation seems to have an interstellar origin and so can be used to estimate the reddening and distance of the nova. Nova Vuleculae 1976 was discovered at about the peak of its brightness on October 21 by the British amateur astronomer Mr Alcock.

Observations were made at the 90-inch (2.3-m) telescope of Steward Observatory on the evenings of October 25–27, 1976 using a dual-channel Pockels cell polarimeter with GaAs photomultipliers (RCA C31034A). Six passbands from 0.35 to 0.86 μm were defined with either interference filters or combinations of coloured glass filters and the spectral response of the photomultipliers. The details and results are given in Table 1 and Fig. 1. Quoted errors are 1σ from photon counting statistics.

The polarisation seems to have an interstellar, rather than intrinsic, origin. The wavelength dependence of linear polarisation is descrbed well by Serkowski's empirical formula for interstellar polarisation[1], with the parameters $p_{\max}$ = 3.35% and $\lambda_{\max}$ = 0.59 μm as shown in the figure. Furthermore $\lambda_{\max}$ and the position angle 12° (72° galactic) are typical for stars in this region[1,2]. There is no evidence for (intrinsic) circular polarisation larger than 0.08% (3σ). Interstellar circular polarisation, however, is likely to be quite small; for example the circular polarisation of HD183143, a more strongly linearly polarised neighbouring

Fig. 1 Linear polarisation of nova Vulpeculae 1976. Curve is empirical formula for interstellar polarisation[1] with $p_{\max}$ = 3.35% and $\lambda_{\max}$ = 0.59 μm.

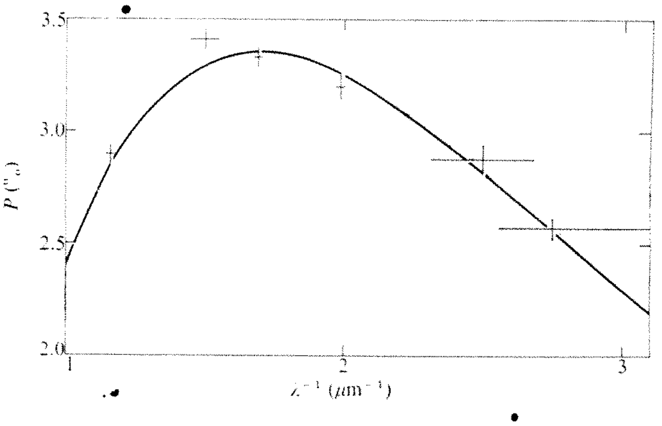

Table 1 Polarisation of nova Vulpeculae 1976

Date (Oct. 1976)	λ^{-1} ($\Delta\lambda^{-1}$ μ^{-1})	Polarisation (%)		
		Linear	Position angle	Circular
25	2.75 (0.57)			0.03±0.02
25	2.75 (0.57)	2.57±0.04	12.2±0.5	
27	2.50 (0.37)	2.88±0.06	10.1±0.6	
26	1.99 (0.03)	3.20±0.06	11.2±0.5	
26	1.69 (0.03)	3.33±0.04	11.1±0.3	
27	1.50 (0.11)	3.41±0.04	12.5±0.3	
26	1.16 (0.03)	2.90±0.04	13.8±0.4	
27	1.16 (0.03)			0.02±0.02

star, is only about 0.01% (ref. 3).

If an interstellar origin is accepted, then the amount of polarisation may be used to estimate the reddening, the distance and thus the absolute magnitude (at maximum) of the nova. Hall[2] lists the colour excess E_{B-V} and the degree of polarisation p (at 0.45 μm) for about two dozen stars within several degrees of the nova. The subset of these that are polarised about the same amount as the nova give p (%) $\simeq$(3.5±1.2 E_{B-V}. For the nova p(0.45 μm)=3.1%, so that the estimated E_{B-V} is 0.9±0.3.

The same subset of stars range in distance modulus from 8.8 to 12.2, the average being about 11. The colour excess in this area of the sky is known[4] to increase linearly with distance to 0.9 at 1 kpc, after which there is little further increase. Therefore a distance modulus of 11±1 for the nova would be consistent with all of the data, though a larger value is possible.

The above estimates, together with a ratio of total to selective extinction of 3.3 and the reported m_V=6.5 near maximum[5], lead to $M_{V,max}$=−7.5±1.5, a reasonable value for a nova. This estimate should be compared to that which can be derived from the timescale of the decay of the nova light, when the latter becomes available.

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Radio 'jets'

FANAROFF and Riley[1] classified extragalactic radio sources according to whether high brightness radio hot spots are observed close to their outer extremities. A change in morphology is found when going from high (typically 10^{26} W Hz^{-1} sr^{-1}) to relatively low (10^{24} W Hz^{-1} sr^{-1}) luminosity at 178 MHz. The more luminous sources have pronounced outer hot spots and are all highly collimated double sources such as Cygnus A. On the other hand, their fainter cousins, which are less commonly found in radio surveys, contain no outer hot spots and comprise sources with relaxed double structure or having head–tail or complex morphologies. So far, little is known how these apparently different classes[1] of sources are related. In particular, one might enquire whether the collimating process is less efficient in the nuclei of the fainter, more relaxed radio sources. Here we present new observations made with the Westerbork Synthesis Radio Telescope (WRST) which show that three of these relaxed type sources with intermediate 178 MHz luminosities have collimated structures emanating from their radio cores. Our high resolution observations show that these coherent structures are embedded in the otherwise smooth radio sources. These 'radio jets' may be similar in nature to the narrow structures recently observed (with less sensitivity) in the collimated double source 3C219 (ref. 2) and the complex radio galaxy 3C66B (ref. 3). We shall briefly discuss the consequences of these radio jets for models of radio sources.

Table 1 Observing parameters

Source	Observing frequency (MHz)	Observing dates	Half-power beamwidth (RA×dec) (arc s)	Effective noise at field centre (10^{-29} W Hz^{-1} m^{-2})
B0844+31	4995	14 Jan. 76 16 Jan. 76	6×12	0.8
	1415	1 July 76	22×42	1.0
	610	Dec. 72– May 73	52×98	4.6
3C310	4995	4 May 73 18 May 73	6×14	0.8
	1415	14 May 71	22×50	1.0
3C129	4995	Dec. 72– Feb. 73	6×9	0.6

New observations were made at 5 GHz of the relaxed double galaxies B0844+31 and 3C310 and of the head–tail galaxy 3C129. B0844+31 was originally classed as a QSO but the high resolution observations of Grueff and Vigotti[4] showed that this was a misidentification. In fact the source is associated with a 15.5 magnitude giant elliptical galaxy[5]. The morphology of 3C310 was discussed by Miley and van der Laan[6] and 3C129 is one of the most studied head–tail galaxies[7,8].

The Westerbork Telescope is described elsewhere[9,10] and relevant parameters pertaining to the present observations are shown in Table 1. Contour maps of the 5 GHz total intensities are shown in Figs 1–3 side by side with lower resolution Westerbork maps made at 1.4 GHz and

Table 2 5-GHz flux densities (10^{-29} W Hz^{-1} m^{-2}) of jets and other features in the sources

Source		Jet	Extended	Core	Total	Redshift
B0844+31	North	75	44	58	250*	0.0675(5)
	South	28	45			
3C310	North	40	1045	95	1280†	0.0540(11)
	South	100				
3C129	North	260	1626	14	2160†	0.0210(11)
	South	290				

*Estimate from fringe amplitudes at shortest baseline.
†Single dish observations (ref. 21).

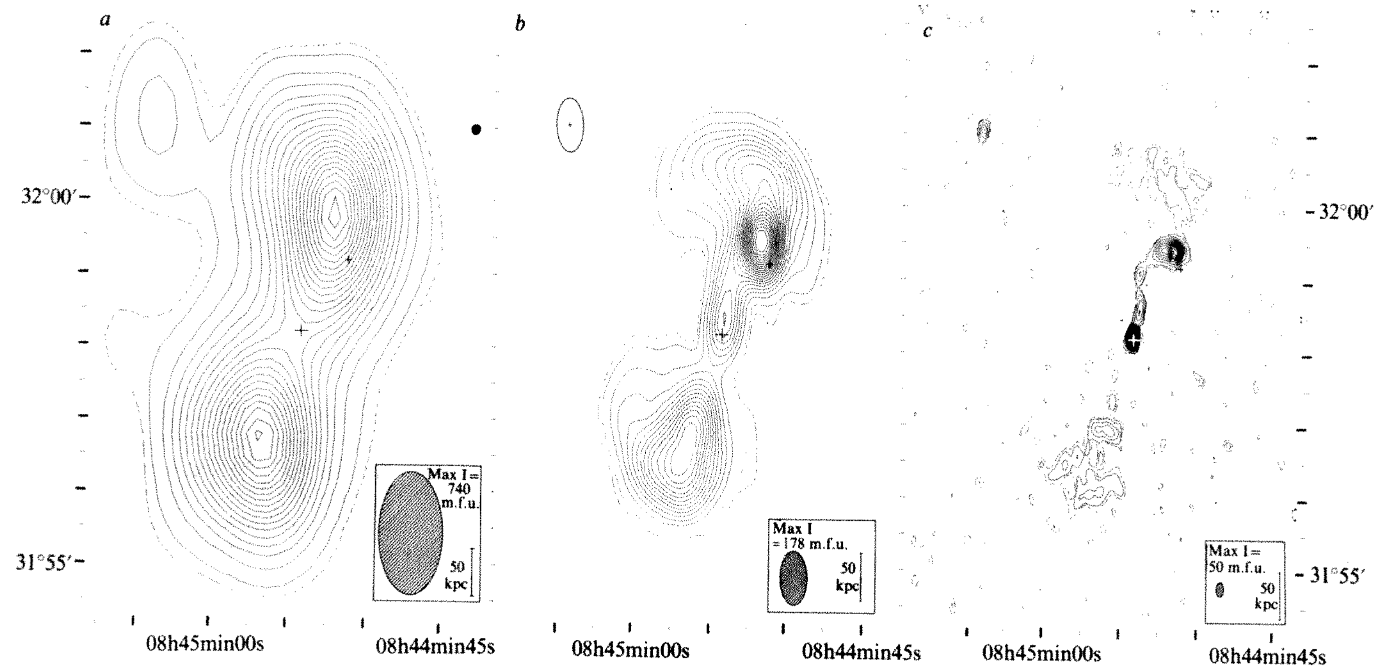

Fig. 1 Total intensity distribution of B0844+31 at 0.6, 1.4 and 5 GHz. Continuous contours are plotted at 5% intervals from 5% to the indicated value of MAX. I. Long dashed contours represent the 3% level and short dashed contours the −5% level. The shaded ellipse represents the half-power level of the synthesised beam and the crosses the position of the galaxy and the QSO. In *b*, an unresolved (presumed background) source is subtracted. Also indicated are the relevant linear scales.

(in the case of B0844+31) at 0.6 GHz. All maps are corrected for primary beam attenuation. The linear scales were obtained using the redshifts listed in Table 2 and a Hubble constant of 75 km s^{-1} Mpc^{-1}. The cross at the central radio component indicates the identified galaxy. For B0844+31, the QSO (at the edge of the northern hot spot in Fig. 1*c*) is also indicated as well as a subtracted (presumed background) point source in the north-east part of Fig. 1*b*.

Note the relaxed appearances of B0844+31 and 3C310 apparent in the lower resolution maps (Figs 1*a*, *b* and 2*a*). By contrast, the 5 GHz observations show a considerable amount of small scale structure. Both sources contain compact radio cores with relatively flat radio spectra coincident with the visible galaxies. Most remarkable are the highly collimated elongated structures emanating from these cores. This is most pronounced in the case of B0844+31 but present to a lesser extent in the southern part of 3C310. From the latter source, however, another characteristic seems to show up more clearly: comparison of Fig. 2*a* and *b* suggests that the coherent, elongated structure is embedded in a larger region of low brightness.

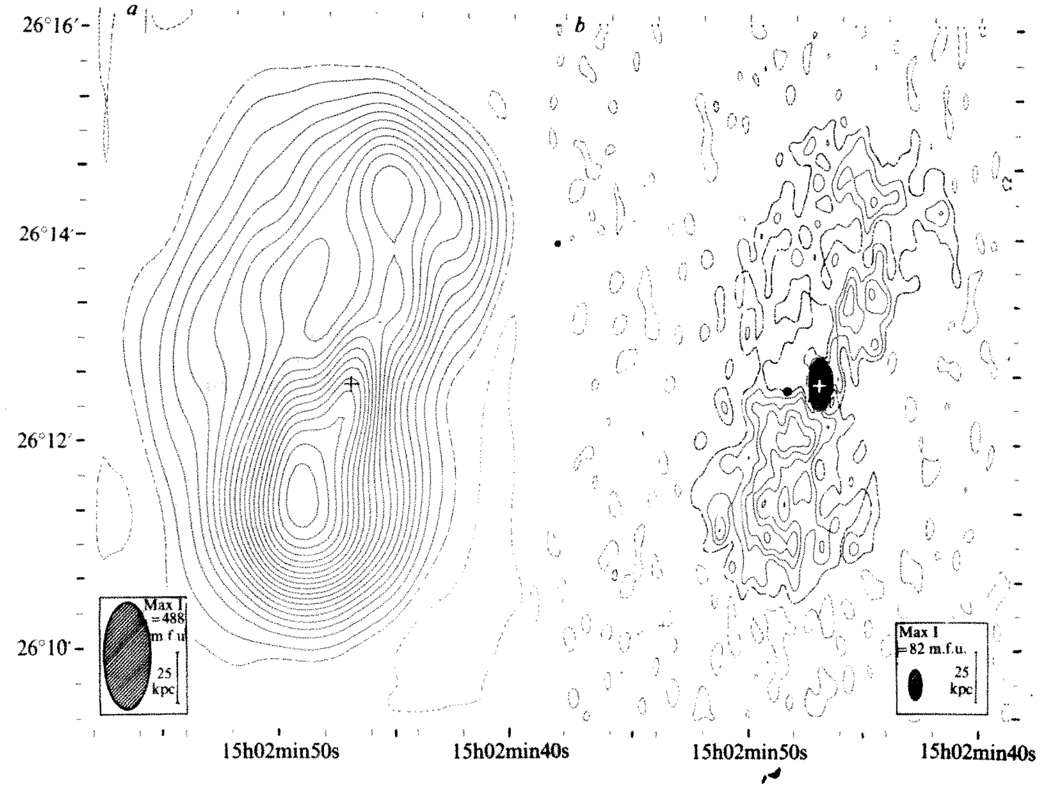

Fig. 2 Total intensity distribution of 3C310 at 1.4 GHz and 5 GHz. Low intensity contours are 2, 5 and 10% in *a* and from 2 to 10% in intervals of 2% in *b*. Further contours are as in Fig. 1. Negative contours are −2% (short dashed). The +2% contours are long dashed.

The radio jet in the northern part of B0844+31 terminates in a region of relatively high brightness situated about half way to the edge of the source. A weaker hot spot is apparent to the south. Beyond the hot spots the emission is relatively uncollimated.

Although the jets are very narrow they are not straight. One can distinguish 'wiggles' on several different scales. For both sources the shape of the wiggles is suggestive of an S-type symmetry[11] close to the radio cores. However, from Fig. 1*b*, the outer parts of the northern and southern components of B0844+31 have relatively sharp gradients to the west and tail off gradually to the east so that the large scale structure of this source may exhibit a C-type symmetry[11].

It is interesting to compare the morphologies of these two relaxed double sources with that of the head-tail source 3C129. Figure 3 shows an unpublished 5 GHz map of the head made with the WSRT. Again there is a compact radio core near the centre of the visible galaxy. According to the radio trail hypothesis[12] the head-tail morphology is formed when a 'normal' double radio source is distorted by the motion of its parent galaxy through a dense intergalactic medium. Without this distortion one might expect the head of 3C129 to resemble the central part of B0844+31. The pair of highly elongated arms stretching back to the north-west are then analogous to the radio jets in Figs 1 and 2. The set of parallel wiggles in the two arms of 3C129 would correspond to the S symmetry in the jets of the relaxed double radio sources. (In the case of 3C129 these wiggles can be traced a distance of several hundred kiloparsecs along the tail.)

Other possible examples of radio galaxies with jets are 3C83.1 (NGC 1265) (ref. 13), 3G130 (ref. 14), 3C402 (ref. 6), 3C449 (ref. 14), 3C465 (ref. 6), Virgo A (ref. 15), Centaurus A (ref. 16), and NGC 315 (ref. 17). When most of these relatively relaxed sources are observed with sufficient sensitivity and linear resolution better than a few tens of kiloparsecs, jet-like features are seen protruding from their radio cores into more extended, low brightness regions. This indicates that although these sources are relatively relaxed, they still have a very efficient collimating process in their nuclei.

Table 2 compares the observed flux densities for the jets with those of other features in the sources. An examination of the data available for the sources presented here together with those of possible candidates indicate that the radio jets typically have 5-GHz luminosities of 10^{22} W Hz^{-1} sr^{-1} and sizes of a few tens of kiloparsecs. The contribution of the jet to the total flux is small, so that for beam widths much greater than the jet widths (a few kiloparsecs), their structure is 'smeared out' over the more extended low brightness regions. The detection of the jets therefore requires a combination of sufficient sensitivity and linear resolving power. The collimated Cygnus A type sources are on average about a hundred times further away and with currently available instruments it is impossible to say whether they also have comparable jets protruding from their cores. However, the 2-kpc component within 3C236 (ref. 18) and the jet component in 3C219 (ref. 2) may represent similar but more powerful phenomena.

How are these radio jets to be interpreted within the framework of current theoretical efforts? In recent years a large body of evidence has built up in favour of models of highly collimated double sources in which energy is supplied quasi-continuously from an active nucleus (at the radio core) to the outer components. The energy is transported by a succession of ejected plasmoids or by a relativistic beam. The existence of the jets suggests that such quasi-continuous energy transport also takes place in the weaker relaxed sources. One can envisage the jet as a tunnel along which the energy is channelled. The hot spots at the outer edges of the collimated doubles have their counterparts in the regions of enhanced radio brightness where the tunnels terminate. In these regions the energy density builds up and results in enhanced synchrotron emission. In the less luminous sources the hot spots are found relatively close to the core. Possibly, since less energy is involved, in this case the energy transport is more influenced by conditions within the parent galaxy.

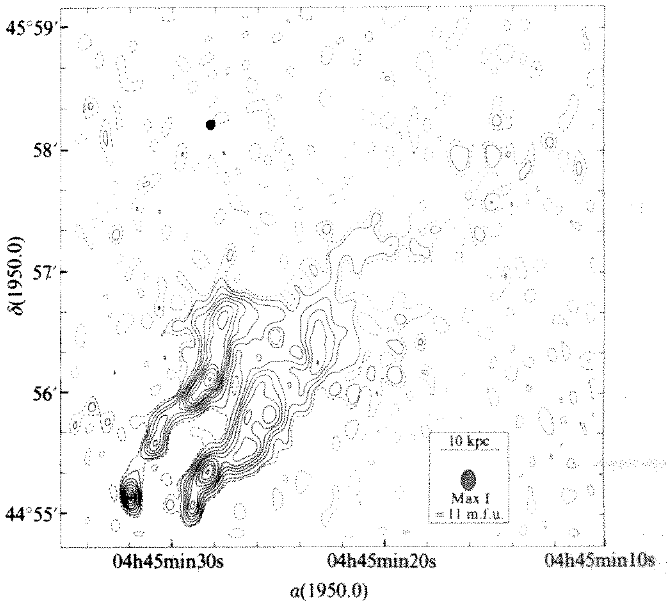

Fig. 3 Total intensity distribution of 3C129 at 5 GHz. Continuous contours are plotted at 10% intervals from 10% to the indicated value of Max. I. A 5% contour (long dashed) and −10% contour (short dashed) are added.

Of particular interest are the S-shaped wiggles in the jets. They appear to occur on a scale about half that of the jets themselves, corresponding roughly to the size of the associated visible galaxies. This correspondence could be indicative of a hydromagnetic instability due to the interaction of the galaxy with the ambient medium. Alternatively the wiggles may be caused by a change in the orientation of the nuclear powerhouse during the source lifetime[19,20]. Such a wobble in the collimation axis could account for the symmetries apparently seen in B0844+31, 3C310 and 3C129 as well as in several other sources[19]. The C-type symmetry in the large scale structure of B0844+31 (Fig. 1*b*) is most probably due to translational motion of the source through an ambient medium.

Analysis of the spectral and polarisation data for these sources may give more insight into the exact role that the jets play in the energy transport process. In the coming year the sensitivity of the WSRT, at 5 GHz, will be improved by a factor of ten. Further effort will then be made to find more sources with radio jets.

We thank our colleagues at Westerbork, Dwingeloo and Leiden who made these observations possible and acknowledge useful discussions with Professor van der Laan. The Westerbork Synthesis Telescope is operated by the Netherlands Foundation for Radio Astronomy with financial support of the Netherlands Organisation for the Advancement of Pure Research (ZWO).

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Frequency of nearby supernovae and climatic and biological catastrophes

McCREA[1,2] has proposed that the encounter of the Solar System with a dense cloud of interstellar material during its passage through a spiral arm of the Galaxy may produce such climatic catastrophes on Earth as the ice epochs. His thesis, an extension of several earlier investigations[3,4,5], is based on the expected effect on the solar constant of an increased accretion rate. Unfortunately, the cloud density necessary to produce the required variation is 10^5 to 10^7 cm^{-3}, and although clouds with such extreme densities are thought to exist[6], Begelman and Rees[7] point out that they are so compact and unusual as to reduce the likelihood of the Sun ever encountering one. (Dennison and Mansfield's suggestion[8] that there is no evidence for the existence of a nearby super-dense cloud which could have caused the most recent ice epoch has been answered by McCrea[9].) Begelman and Rees[7] in fact showed that a much more modest cloud density ($\simeq 10^2$ to 10^3 cm^{-3}) would prevent the solar wind from reaching the Earth, with resulting modification of the near-Earth environment; however, the climate consequences of such a modification are not well understood. We present here an alternative hypothesis which still retains McCrea's association with the passage of the Solar System through a spiral arm, but which relates the initiation of an ice epoch, plus biological catastrophes, to an encounter of the Solar System with a nearby supernova outburst rather than a super-dense interstellar cloud.

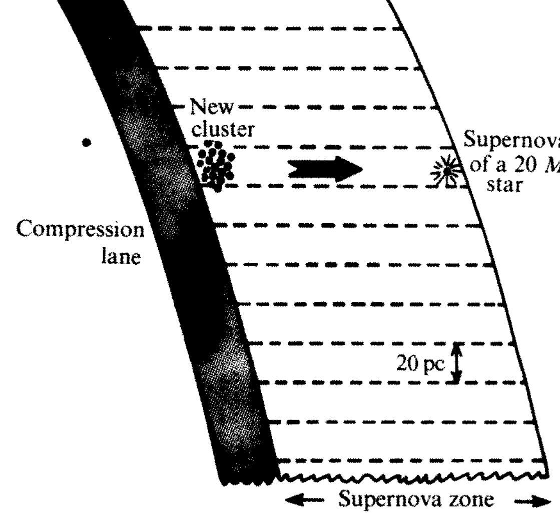
The spiral structure of our Galaxy indicates the regions where bright, short-lived stars illuminate the interstellar gas and dust. The two principal spiral arms are believed to reflect a self-sustaining density-wave pattern through which the disk of stars and gas move. Observations indicate dust lanes along the inner (concave) edge of each arm, and this is interpreted as a shock-wave effect whereby clouds become temporarily compressed as they cross the lane. A cloud of interstellar matter in its orbit round the Galaxy is liable to enter a spiral arm by way of its compression lane about once in $\simeq 10^8$ yr. In general the cloud material will re-expand on emerging from the lane after $\simeq 10^6$ yr (the cloud not necessarily retaining its original identity). But sometimes a cloud is compressed to a condition for star formation, and the result is a new galactic cluster which proceeds to traverse the associated spiral arm itself, taking $\simeq 10^7$ yr to do so. If this cluster contains a star of mass some $10 M_\odot$ or greater it would be expected to evolve rapidly over less than $\simeq 10^7$ yr, terminating in a supernova outburst of Type II before emerging from the spiral arm in which it is formed. In this view supernovae of Type II would occur only in spiral arms (or near galactic nuclei), in accord with observation. Indeed, a 'supernova zone' might be defined beyond the compression lane of a spiral arm; such a zone is depicted diagrammatically in Fig. 1, in the assumption of a $20 M_\odot$ stellar lifetime of $\simeq 5 \times 10^6$ yr and a transverse velocity (at about the solar distance from the galactic centre) of 200 km s^{-1} giving a zone width of $\simeq 1$ kpc.

The occurrence rate of supernovae within the Galaxy is still only poorly determined. At least five historically observed supernovae occurring on the near-side of the Galaxy during the past millennium[10,11] would seem to place a lower limit on their frequency throughout the Galaxy of about one per 100 yr. It must, however, be stressed that the short interval between some actial sightings of supernovae can give a grossly misleading impression of the frequency of events because of the extreme distances involved. For example, the AD 1006 supernova occurred at a distance of about 3,000 light years from the Earth[12], while that of AD 1054 occurred at a distance of over 6,000 light years (ref. 12), so that although first sighted on Earth within half a century of each other the outbursts were actually separated in time by over 3,000 yr. Clark and Caswell[13] have concluded from a statistical analysis of the radio remnants of supernovae that the average interval between outbursts leaving long-lived observable remnants (that is, those lying within spiral arms and evolving only slowly) is $\simeq 100$ yr. Shklovsky[14] prefers an interval of a mere 30 yr for Type I (population 2) events, but accepts a longer interval of $\simeq 100$ yr for those of Type II (population 1). Results from other investigators range between these extremes. In the absence of a more define estimate, we will take an average interval between Type II supernovae of 100 yr.

If there is one Type II supernova per 100 yr, and if, as seems reasonable, this is localised to one of the 'supernova zones' proposed above, then this implies 5×10^4 such supernovae in one (of two) spiral arms in a period of $\simeq 10^7$ yr (the time for the Sun to traverse a spiral arm). Since a spiral arm is $\simeq 2 \times 10^4$ pc long, this suggests that 50 supernovae will occur in a 20-pc strip of a spiral arm's 'supernova zone' each time the Sun crosses it. Thus at least one supernova would be expected to occur within 10 pc of the Sun each time it crosses a spiral arm (that is, about once every 10^8 yr). Note that this simple calculation takes no account of the thickness of a spiral arm perpendicular to the plane; however, since the bulk of supernova remnants are concentrated to within a few tens of pc of the plane[13], this simplification is not expected to increase the above estimate for the interval between nearby supernovae by more than a factor of two.

Estimates of the radiation flash[15] emitted (mainly in γ and X rays) in a supernova explosion vary from $\simeq 10^{47}$ to 10^{50} erg. (This represents a very small fraction of the total energy of the outburst of $\simeq 10^{52}$ erg.) Assuming the lower value, the peak integrated energy flux expected above the Earth's atmosphere from a supernova at $\simeq 10$-pc distance is $\simeq 10^6$ erg cm^{-2} (this compares with the solar flux of $\simeq 20$ erg cm^{-2}s^{-1} in the wavelength range of interest). Absorption in the upper atmosphere means that this flash gives no significant surface radiation dose initially. Nevertheless, Ruderman[15] has predicted catastrophic effects on the Earth's protective ozone layer. The ionising radiation from the supernova

Fig. 1 Schematic representation of a supernova zone (not to scale).



flash produces excited free nitrogen atoms in the atmosphere which quickly oxidise. The oxides of nitrogen catalytically destroy ozone, and Ruderman claims that by this mechanism the ozone layer density may be temporary depleted. Such destruction of the ozone layer would expose the Earth's surface, and more particularly life on it during the past 600 Myr, to lethal solar ultraviolet radiation.

The expanding shell of ejecta from a supernova at 10-pc distance would reach the Earth after about 2,000 yr, with the passage of the shell taking several hundred years. During this latter period the density of primary cosmic rays would be expected to be enhanced by perhaps a factor of a hundred[14]. About one third of the background radioactivity level at the Earth's surface is contributed by cosmic radiation (the remainder being due to terrestrial factors). Thus a hundredfold increase in cosmic ray intensity would produce a drastic thirtyfold increase in mean level of radioactivity. In addition, the enhanced cosmic-ray flux would be expected to produce the same destruction of the ozone layer as produced by the initial radiation flash. The possible biological consequences of such dramatic increases in background radioactivity and ultraviolet radiation fluxes expected for protracted periods some time after a nearby supernova outburst may have been catastrophic for certain species. Ruderman[15] has suggested that in man the huge increase in ultraviolet radiation would give rise to a vast increase in the incidence of skin cancer; in animals with calcified internal skeletons the increased vitamin D production might have been toxic.

The supernova-initiated destruction of the ozone layer would be expected to have climatic as well as biological consequences. Whether ozone removal would heat or cool the Earth is unclear; the main argument for cooling is that ozone contributes (in the 2-μm region) significantly to the greenhouse effect, so that its removal could lower the Earth's temperature and thus possibly initiate an ice age.

The question arises as to whether one would be able to observe the nearby remnant of the supernova which might have initiated the last major ice age $\simeq 10^5$ yr ago. Unfortunately, after such a period the remnant of this supernova would already have reached its extinction phase[13], and would almost certainly be impossible to identify with certainty.

The possible astronomical influences on the Earth's climatic and biological history must remain a matter of speculation. Nevertheless, we suggest that the evidence that the Solar System encounters a nearby supernova (that is closer than 10 pc) on average once per orbit of the Galaxy must be included in any future consideration of these topics.

Note added in proof: In a recent note, Whitten *et al.*[17] have independently considered the effects of a nearby supernova on atmospheric ozone. From a consideration of the galactic distribution of supernova remnants given by Clark and Caswell[13], they concluded that the probability of a supernova occurring within 10 pc of the Earth during the past 10^8 yr is only 1–5%. Our somewhat greater estimate of a 50% probability during any 10^8-yr period is a consequence of our confining (at least Type II) supernovae to the galactic spiral arms, in accord with the observational evidence[12,14], and assuming that within the spiral arms the rate of occurrence of supernovae is independent of distance from the galactic centre.

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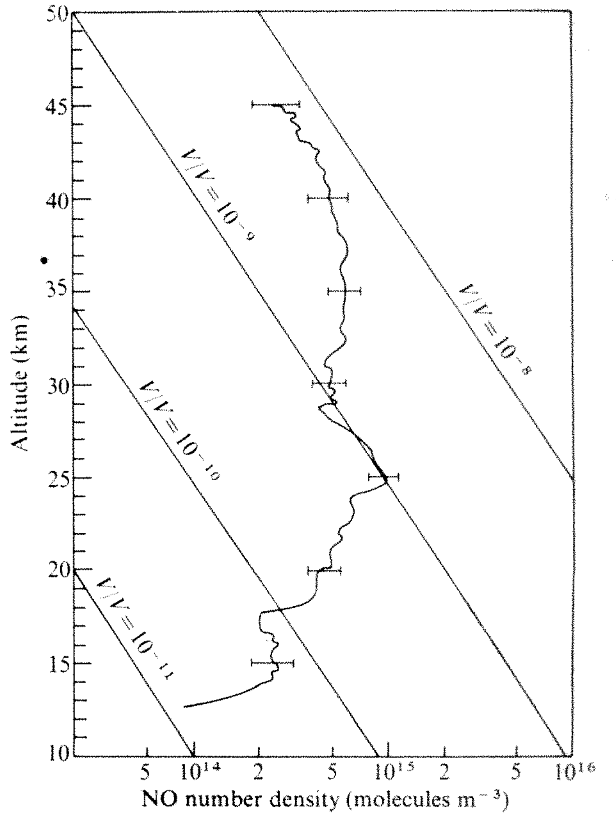
Balloon-borne chemiluminescent measurement of NO to 45 km

MEASUREMENT of nitric oxide (NO) above an altitude of 40 km is of interest because in this region of the stratosphere >70% of the total odd nitrogen ($NO+NO_2+HNO_3+N_2O_5$) is thought to exist as NO, and at 45 km the NO is predicted to be approximately 90% of the total[1]. This measurement thus provides an important test for odd nitrogen concentrations as predicted by theoretical models for ozone chemistry. Some models show that of the total ozone destruction necessary to explain the observed ozone concentration, approximately 50–70% is due to the odd nitrogen catalytic cycles[2].

We have made direct measurements of the nitric oxide (NO) concentration between 12 and 45 km with a balloon-borne chemiluminescent detector. This is the first balloon-borne chemiluminescent measurement to an altitude of

Fig. 1 Vertical profile of the nitric oxide concentrations for the June 27, 1976, balloon flight from Sioux Falls, South Dakota (1 atmosphere 43°26′N). The instrument was launched at 1108 GMT and the data were taken on ascent. The local Sun times for the data at 12 km and 45 km were approximately 0530 and 0730, respectively. The marked increase in NO at 18 km correlated with the altitude of the stratospheric wind reversal. The altitude of the tropopause was 10.5 km.



45 km. The data above 40 km are particularly useful to test theoretical models of ozone chemistry because above 40 km most of the odd nitrogen is thought to be in the form of NO. The data from a balloon flown on June 27, 1976, from Sioux Falls, South Dakota (Fig. 1), show a nearly constant mixing ratio of 6.5 p.p.b.v. from 40 to 45 km, with a possible peak value at 43 km. One other high-altitude stratospheric NO measurement has been made by a rocket-borne chemiluminescent detector but the data were taken quickly (in 25 s) and without on-board flow measurements, zeroing, or calibration[3]. Several chemiluminescent devices, interferometers, spectrometers, and lasers have been flown on balloons to a maximum altitude of 35 km (refs 4–7). These measurements indicate a large range in NO concentrations which suggests that transport plays a strong role for odd nitrogen and thus ozone chemistry in the lower stratosphere.

Our instrument was similar in principle to other chemiluminescent NO detectors[8], and a brief description of the device follows. Ozone for the photon producing reaction $NO+O_3 \rightarrow NO_2+h\nu$ was carried as ozone absorbed on silica gel at dry ice temperatures. The O_3 source was servoed to produce a constant concentration of O_3 in the reaction vessel at all ambient pressures. The sample gas was drawn through a 1.2-m pyrex tube and heated to 30 °C before entering the gold-plated reaction vessel. A positive displacement blower was specially developed to provide a constant 3 l-s^{-1} flow rate at all altitudes encountered during the flight. The blower exhausted the sample gas–ozone mixture through a 3-m flexible tube located on the side of the instrument opposite the sample inlet tube. The balloon ascent provided a 15-km-h^{-1} vertical flow rate so that the exhausted ozone did not pollute the sample. The instrument was suspended 100 m below the balloon and flight control package. Zero NO signal levels were taken every 40 s by destroying the NO in the sample gas with ozone upstream of the reaction vessel. The instrument was calibrated every 2.5 min by pumping NO (in N_2) calibration gas into the sample inlet tube.

The data for the ascent are shown in Fig. 1. The mixing ratio from 40 to 45 km is essentially constant at 6.5 p.p.b.v. with a possible peak at 43 km. The results show considerable structure in the NO concentration in the lower stratosphere. There is a marked increase in NO mixing ratio at 18 km which corresponds to the altitude of the wind reversal for that day. Where data can be compared, our results show general agreement with other measurements[1,9].

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Sea surface temperature related to rain in Ceará, north-eastern Brazil

RAINFALL in Ceará is correlated with sea surface temperature in the south Atlantic Ocean. Knowledge of sea surface temperature makes possible a useful rainfall forecast before the rainy season begins. The mechanism may be that sea temperature affects the height of the trade wind inversion, and thus the height of the moist layer. The data also suggest in association between below normal sea temperatures in the south Atlantic and the El Niño in the Pacific.

Ceará is the node of Brazil's drought-prone north-east. Here severe drought, and its antithesis, flooding, both occur about once per decade, arriving without warning. The human misery that follows in their wake might be reduced if they could be forecast. We found that sea surface temperature (SST) in the south Atlantic Ocean and rainfall in Ceará are correlated in time, and if SST is known, a useful preliminary rainfall forecast can be made.

Brazilian climatologists have long believed rain in the north-east to be related to fluctuations in SST, but have been unable to show such a relationship owing to lack of sufficient SST reports. We investigated this relation by using a data file containing all available surface marine weather reports from ships of many nations. This file, Tape Data Family -11, was assembled by NOAA's National Climatic Center. Working in conjunction with the Fleet Numerical Weather Central and the North Pacific Experiment (NORPAX) the National Marine Fisheries Service has used the tape data file to compute mean sea surface temperatures by 5° latitude-longitude quadrants for each month in the period October 1947 to December 1967. We used these computations to map the correlation coefficient between SST and a rainfall index in Ceará. Later, after we had identified a key 5° quadrant where correlation was particularly high, monthly SSTs were recomputed for the period 1907–72, and used in a regression analysis to develop a forecasting equation. Our results may be somewhat biased by the selection of this particular quadrant, and further research may show that improved forecasts can be made using data from other areas.

The density of SST observations is far from uniform over the South Atlantic. Reports are most abundant along shipping lanes between the Cape of Good Hope and Cape Verde, and along the South American coast. Data are extremely sparse over the central South Atlantic, and are not sufficient to map correlation coefficients over the entire area. Data are not available in all quadrants in all years, and different years are missing from different quadrants. Nevertheless, when considered as a whole, the data form a picture that is coherent and of predictive value.

The rains in Ceará are highly seasonal, about 40% coming in January-February-March, and about half in April-May-June. Almost no rainfall occurs in the months August to November. Fortaleza and Quixeramobím are two weather stations in Ceará about 120 km apart that have long series of rainfall observations extending back to 1849 and 1896, respectively. We correlated monthly mean SST data with rainfall at both stations, but found that a combined index of simultaneous data from both stations gave higher correlation coefficients. The combined index smooths local variations of rainfall to emphasise large scale influences affecting both stations. Since the mean annual rainfall at Fortaleza, on the coast, is about twice that of Quixeramobím, in the interior, we gave each station equal weight in the index by using twice Quixeramobím's rainfall plus Fortaleza's.

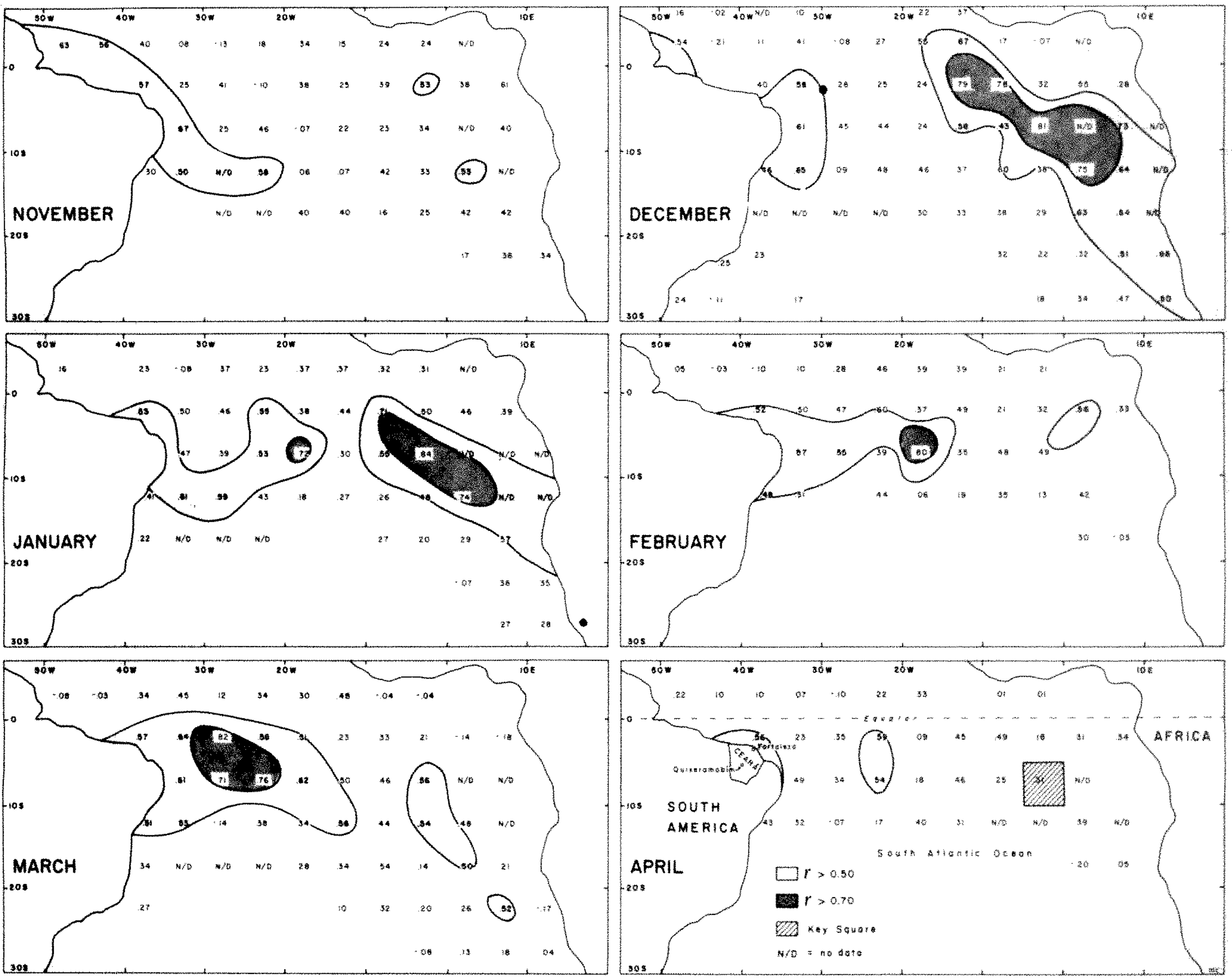

Fig. 1 Correlation between SST and January-February-March rainfall indices in Ceará. An area of high coefficient of correlation appears off the African coast in November, becomes strong in December, moves westward with the South Equatorial Current during January, February and March, and disappears in April.

We computed correlation coefficients (r) for each of the 5° quadrants in the South Atlantic for which data are available, between the monthly values of SST and the rainfall index for the combined months of January-February-March, as well as for the annual rainfall indices. SSTs for November and December were correlated with rainfall indices for the coming year, while SSTs for January through April were correlated with the current year.

Maps of correlation coefficients (Fig. 1) show an apparent westward movement of high correlation during November to April. This may be related to the westward drift of water in the South Equatorial Current. In December the quadrant where correlation is highest is centred at 7°30′S, 2°30′W (Marsden Square 300, Quadrant 3). For this area r was 0.81 for January-February-March rainfall, and 0.73 for annual rainfall. Ceará tends to be dry when water here is cold, and wet when it is warm. Moreover, since December precedes the rainy season in Ceará, December SST can be used to forecast rainfall with useful lead time. Thus we selected Quadrant 300-3 as the key area for regression analysis with Ceará's rainfall.

The 1947-67 data used for our correlation maps are means of all reports within a given 5° quadrant and thus might be biased by clustered reports. This possible bias can be reduced by making individual means for each 1° area, then averaging the 1° means to obtain a 5° mean. This latter procedure was used to obtain data points for our long term regression analysis (Fig. 2).

We noticed a long term warming trend of 0.51 °C between the years of 1907 and 1972, in our SST means at Quadrant 300-3. The trend could be caused by real warming, or by differences in measurement techniques that have occurred over the period. If it is assumed that the latter is true, and if the effects of the trend are removed from the regression, r for the period increases from 0.61 to 0.66, Kendall's rank-order coefficient, τ increases from 0.20 to 0.41. This increase in order through detrending suggests that the apparent trend is instrumental and not a real warming.

Depending on the sets of data, r values range from 0.61 in the 1907–72 data uncorrected for trend, to 0.81 in the 1947–67 data used in making the correlation maps. Values of τ range from 0.21 to 0.50. All are for correlation between December SST and the Ceará rainfall index for the following January-February-March period. If we assume r to be 0.71, then r^2, the coefficient of determination, is 0.50, indicating that half of Ceará's January-February-March rainfall can be predicted by mean SST in Quadrant 300-3 in December. If we assume $\tau = 0.40$, we can say that the odds are 70/30 that a change in SST will be followed by a change in rainfall index of the same sign. Our rainfall forecast ranges from 500 mm with an SST of 22 °C to 2300 mm

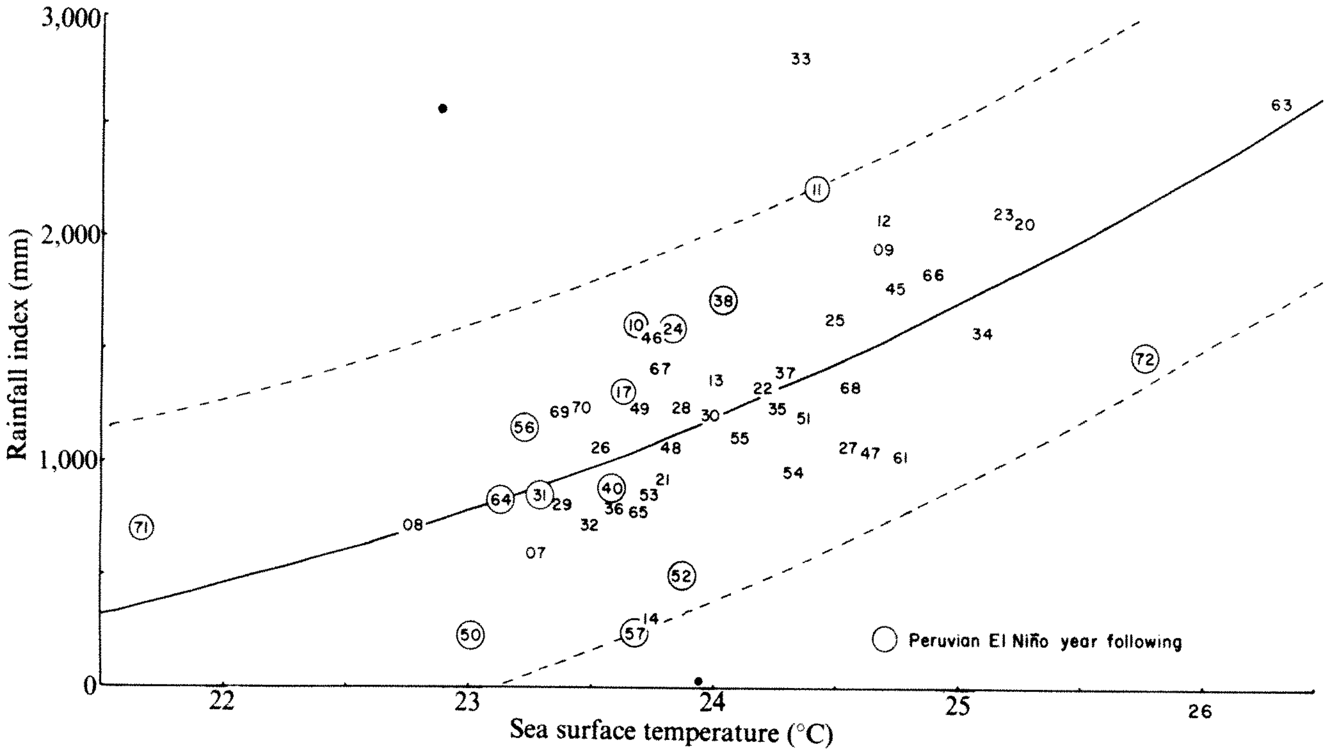

Fig. 2 January-February-March rainfall indices in Ceará plotted against December SST in Marsden Square 300, Quadrant 3. Years indicated are for December SST values. Data has been detrended by adding 0.0078 °C times the number of years before 1972. Second degree regression lines and 95% confidence limits are shown. El Niño years (taken from Caviedes (1973) and Quinn[6]) are circled.

	1907–72 Detrended	1907–72 Not detrended	1947–67 From Fig. 1
n	53	53	16
r	0.66	0.61	0.81
α	0.001	0.001	0.001
τ	0.41	0.20	0.50
α	0.001	0.04	0.007

with an SST of 26 °C; a considerable improvement over the climatic normal value of 1267 mm. If we define drought as the lack of rainfall and flooding as an excess of it, from Fig. 2 we can state that drought is unlikely when SSTs at Quadrant 300-3 are warmer than 24.5 °C, and that flooding is unlikely when SSTs are colder than 23.5 °C. If we are given some particular SST value, say 24.0 °C, our best estimate for the rainfall index is 1200 mm, and we can say with 95% confidence that it will fall between 350 and 2050 mm. These are much wider limits than we should like but suggest that eastern Atlantic SST in December, albeit important, is only one of a number of factors influencing Ceará's rainfall.

A suggested mechanism for the correlation is as follows: cold water stabilises the overlying air and decreases wind flow and convection over the Atlantic. The trade wind inversion represents the boundary between subsiding air from aloft and convective air from below. When convection is weak and winds are light, the trade wind inversion is lowered, and the thickness of the layer carrying moisture into north-eastern Brazil is reduced. Similarly, warm seas strengthen wind flow, and mixing and increased convection raise the trade inversion to deepen the moist layer. Further, the Intertropical Convergence Zone (ITC) and its attendant raininess would tend to lie in the region of warmest water, and would retreat from the hemisphere with cold water.

Namias[1] found intense cyclonic activity near Newfoundland to be highly correlated with rain at Quixeramobim. He hypothesises the following chain of events. The North Atlantic Subtropical High is strengthened when the trough in the Newfoundland land area deepens. The trough is held in place by blocking toward the east. The strengthened subtropical High increases the north-east trade winds which in turn require the south-east trades to respond by strengthening, thus increasing the flow of moisture into Ceará. An alternative explanation for Namias' correlation is its reverse. Warm South Atlantic sea temperatures produce heavy equatorial rains. These release large quantities of latent heat, strengthening the Hadley circulation in both hemispheres, and inducing a blocking High in the North Atlantic. The increased flow from the Equator, accompanied by the blocking, results in increased cyclonic activity in the Newfoundland area, with a concurrent deepening of the trough. To support this latter explanation, Markham[2] found that a strong South Atlantic High and strong south-east trades seem to decrease, rather than increase, rain in Ceará. The rainiest periods do not occur when the south-east trades are strong, but when the High is weak or has retreated toward the south, allowing flow with a northerly component to penetrate Ceará. There is little doubt that the Newfoundland trough and rain in Ceará related, but which is the cause and which the effect is unclear. In any event, precipitation over northeastern Brazil is important to the general circulation because it represents a significant energy input to the atmosphere, and with large variations from year to year. The magnitude of this variation is shown by the annual rainfall index which varied from a low of 884 mm in 1915 to a high of 5505 mm in 1964.

From the work of Namias[3] and Bjerknes[4], a body of literature on large scale ocean–atmospheric interactions is growing. Recently Caviedes[5] has related drought in northeastern Brazil with the El Niño—the appearance of anomalously warm water off the Peruvian coast. Temperatures in Quadrant 300-3 also seem to be related to El Niño. The years preceding El Niño are circled in Fig. 2.

The hypothesis that cold December SSTs precede El Niño has a one-tailed significance level of about 0.0125 when the non-parametric Wilcoxson two-sample test is applied[7]. The coldest December temperature in Quadrant 300-3, 21.67 °C in 1971, was followed by an El Niño of exceptional severity in 1972. The El Niño of 1972 may have persisted into 1973 and thus may account for the anomalous point for 1972 circled in Fig. 2. If this did occur, the correlation is greatly improved. Quinn[6] has related El Niño to the difference in surface pressures between Easter Island and Darwin, Australia. This difference is an index of the Southern Oscillation and suggests that the occurrence of warm water off Peru simultaneously with cold water off Africa is related to the Southern Oscillation. The mechanisms for this phenomenon are poorly understood.

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Upper Jurassic pyroclastic rocks in Skye, west Scotland

DURING a systematic sampling of shale lithologies in the Jurassic beds of Skye, an examination was made of sections in the Staffin Shales[1-3] in Staffin Bay. Two localities were visited, one immediately south of the landslip at point 6 (ref. 4) [NG470712] and the other at point 7 (ref. 2) [NG468715]. Six shale samples were collected at the former section, representing the *Kosmoceras jason* and *Erymnoceras coronatum* zones (Middle Callovian), the *Peltoceras athleta* and *Quenstedoceras lamberti* zones (Upper Callovian), and the *Quenstedoceras mariae* and *Cardioceras cordatum* zones (Lower Oxfordian). Four shale samples were collected from the section at point 7, representing the *Ringsteadia pseudocordata* zone (Upper Oxfordian) and the *Pictonia baylei*, *Rasenia cymodoce*, and *Aulacostephanus mutabilis* zones (Lower Kimmeridgian).

The shale samples were thus representative of the bulk of the sequence, but further samples were collected from two of three distinctive pale grey mudstone bands present in the point 6 section. The lowest of these mudstones (here termed the *athleta* band) lies about 0.75 m above the base of the shales of the *Athleta* zone. The upper two bands, about 1.7 m apart, could not be zonally dated from field observations alone, but foraminiferans from the lower band have been identified by Dr A. W. Medd as belonging to the lower part of the *Cordatum* zone (*Bukowskii* or *Costicardia* subzone) of the Lower Oxfordian. As the upper part of the *Cordatum* zone (*Cordatum* subzone) is at least 4.8 m thick[1], the upper band must also belong to the *Cordatum* zone. The upper two mudstone bands are therefore referred to as the lower and upper *cordatum* bands, respectively.

Petrographical examination of the shale samples has revealed the presence of minor amounts of pyroclastic material, as discussed below. More striking evidence of volcanic ash-fall is, however, provided by the mudstone bands. These are internally structureless apart from mottling due to burrows. They shrink and break into small angular fragments on drying at room temperature and on subsequent immersion in water they rapidly swell and disintegrate to a flocculent mass of clay and a residue of fine particulate material. The residual particles consist largely of irregular grains composed of isotropic material, chlorite or calcite. Thin sections reveal a poorly defined normal grading of these grains, some of which display elliptical structures similar to amygdales in basic volcanic glass. Accessory grains include euhedral or fractured-euhedral prismatic apatite crystals and fresh euhedral or angular-anhedral flakes of biotite. By contrast the associated shales contain a typical detrital assemblage of abundant quartz, with minor feldspar, muscovite, chlorite and biotite.

X-ray diffraction analysis shows the clay fraction to consist of pure montmorillonite in the lower *cordatum* band and montmorillonite and kaolinite in the *athleta* band. The clay fractions of the associated shales in both cases consist principally of mixed-layer montmorillonite–illite and kaolinite.

In their physical properties and mineral composition these mudstones are comparable with some of the well-documented American bentonites[5], and there can be little doubt that the mudstones represent tuffs which have undergone diagenetic alteration to bentonitic clays. The absence of quartz and zircon in the residues suggests that the tuffs were not acidic and the absence of attenuated shard structure in the glass particles also implies a basic or intermediate composition. The presence of biotite indicates that the source lavas were at least in part of andesitic or trachytic type. Feldspars are absent in both of the mudstone bands but it is probable that scattered patches of coarsely crystalline calcite represent the replacement of primary feldspar crystals.

Undoubted volcanic material (altered glass particles and euhedral apatite crystals) also occurs in minor amounts in otherwise 'normal' detrital shale samples from the *Coronatum*, *Athleta*, *Lamberti*, *Mariae* and *Pseudocordatum* zones (the *Plicatilis*, *Transversarium* and *Cautisnigrae* zones were not sampled). Altered glass particles also seem to be present in the *Baylei* and *Cymodoce* zones of the Lower Kimmeridgian, although they are too finely grained to display diagnostic amygdaloidal textures. No volcanic constituents have been recognised in samples of the *Mutabilis* zone of the Kimmeridgian or of the *Jason* zone of the Middle Callovian.

Microscopic examination of Bajocian and Bathonian shales collected in the same area has not revealed any recognisable volcanic components, although the abundance of montmorillonite in some beds is suggestive of a volcanic contribution[6]. Thus although volcanism may have contributed finely divided ash to the Middle Jurassic sediments, it would appear that a phase of stronger volcanic influence started in the Middle Callovian and lasted at least until the Lower Kimmeridgian. Phases of maximum activity during the Upper Callovian and Oxfordian are indicated by the altered tuff bands.

Jurassic volcanicity in the continental shelf area around Britain is now well established with the discovery of lavas and tuffs in Middle Jurassic beds of the Piper–Forties area of the North Sea[7]. These volcanics are dated as Bathonian and Bajocian. Another volcanic centre or centres to the south-west of Britain may have been the source of volcanic components of the Bathonian Fuller's Earth of the Bristol district[8], and perhaps also of the montmorillonite bands in the Bathonian of the East Midlands[6]. Upper Jurassic (?Kimmeridgian) tuffs have been recorded from the Brent area of the North Sea[7], and a possible tuff reported in the Kimmeridgian beds of Andøy, Norway[9].

If its inferred Jurasic age is correct, the Piper–Forties volcanic centre cannot have been the source of the Upper Jurassic pyroclastic deposits of Skye, although it is possible that the North Sea eruptions are represented in Skye by the high proportion of montmorillonite in Middle Jurassic beds (ranging from the Dun Caan Shales, Bajocian (Aalenian), *Ludwigia murchisonae* zone to the Ostracod Shales, ?Lower Bathonian).

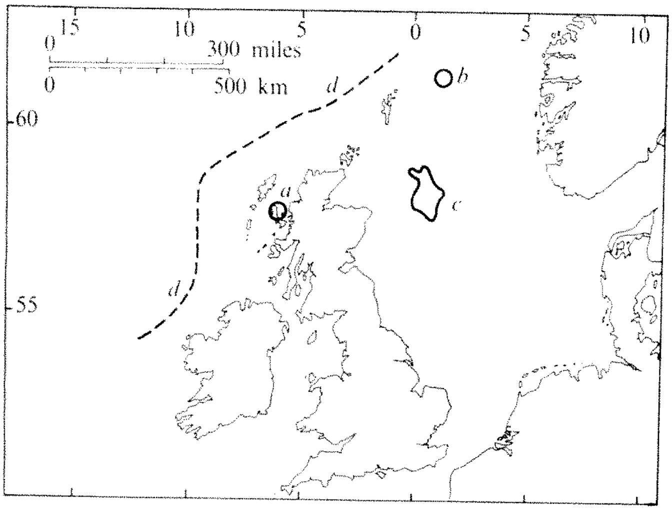

Fig. 1 Location of Upper Jurassic tuffs in Skye (*a*) and in the Brent area of the North Sea (*b*). The Piper–Forties volcanic centre (*c*) is essentially of Middle Jurassic age and Upper Jurassic volcanism may have occurred in a zone of potential rifting (*d*) to the west and north of Britain.

A possible alternative source area for the Upper Jurassic tuffs is the zone now represented by the series of NE–SW troughs[10] which follow the present-day shelf margin to the west and north of Scotland. The closest of these, the Rockall Trough, may not have developed until Cretaceous times but a period of initial rifting and volcanism may have begun as early as the Middle Jurassic[11]. With the limited information at present available it seems most likely that the volcanic source for the Skye and perhaps Brent tuffs lay within this zone of potential late Jurassic rifting (Fig. 1).

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An unusual electromagnetic surface force

IN a recent experiment[1] a disk of high dielectric constant material (barium titanate) was suspended as a torsional pendulum in an intense axial magnetic field. It was found that if a radial electric field was applied to the disk in time quadrature with the magnetic field a substantial torque was produced which, to within experimental accuracy ($<5\%$), was in agreement with a force density $\dot{\mathbf{P}} \times \mu_0 \mathbf{H}$ in the disk. The surprising feature of the torque was that it had a time average, contradicting the 'Abraham force' which predicts a force density of $\partial(\mathbf{P} \times \mu_0 \mathbf{H})/\partial t$ with zero time average. The experiment seemed to suggest that the quantity $\dot{\mathbf{P}} \times \mu_0 \mathbf{H}$ does not produce a force since its time average would be equal but of opposite sign to the time average of $\mathbf{P} \times \mu_0 \dot{\mathbf{H}}$.

In order to obtain a high electric field strength in the dielectric a thin metal coating was evaporated on to the outside edge of the disk and the edge of the central hole. The electrical resistance of these coatings was sufficiently high so that induced currents due to the time-varying magnetic field had a negligible effect on the total electric and magnetic fields. As a check, cuts were made in the coatings, thereby preventing any circulating current flow, with no observable change in the behaviour of the system.

On further investigation it seems that the metal coatings have a most unexpected effect, as may be seen from the following argument. Suppose that air gaps are created between the coatings and the dielectric surfaces, the gaps being small enough to cause no significant perturbation of the electromagnetic fields. Three mathematical closed surfaces can be described, one just outside and surrounding the dielectric and the other two just outside and surrounding the metal coatings. The moment of the electromagnetic surface stress for each surface can be obtained from the Maxwell tensor by evaluating the integral

$$\int \mathbf{r} \times [\varepsilon_0 \mathbf{E}\,(\mathbf{E}.\mathbf{n}) + \mu_0 \mathbf{H}\,(\mathbf{H}.\mathbf{n}) - \tfrac{1}{2}(\varepsilon_0 \mathbf{E}^2 + \mu_0 \mathbf{H}^2)\,\mathbf{n}]\,\mathrm{d}S$$

For the surface enclosing the dielectric it may be shown that this has zero time average but for the surfaces enclosing the metal coatings the moment reduces to $\int \mathbf{r}(\varepsilon_0 E_r) E_\theta \mathrm{d}S$ where the latter is taken over the parts of the mathematical surfaces in the air gaps. When this is evaluated the measured value of torque results. The interpretation is that an electrical charge of density $\varepsilon_{0r} E$ is induced on the surface of the coating adjacent to the dielectric and a drag action is exerted on this surface charge by the azimuthal electric field component E_θ.

In light of this, the experiment verifies that the total mechanical force on the dielectric has no time average, as is consistent with the Abraham force. That the term $\mathbf{P} \times \mu_0 \dot{\mathbf{H}}$ produces a force can be understood by considering the Kelvin force $(\mathbf{P}.\nabla)\mathbf{E}$ which may be written $\frac{1}{2}(\varepsilon - \varepsilon_0)\nabla \mathbf{E}^2 + \mathbf{P} \times \mu_0 \dot{\mathbf{H}}$. In the above experiment $\mathbf{E}$ is independent of θ and so $\nabla \mathbf{E}^2$ does not contribute to the torque. A further conclusion to be drawn is that the experiment is consistent with a force density in the dielectric given by $\dot{\mathbf{P}} \times \mu_0 \mathbf{H} + (\mathbf{P}.\nabla)\mathbf{E}$ provided $\mathbf{E}$ is the macroscopic internal field and not a 'local' field as proposed in different forms by a number of authors.

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Insect growth regulator and sterile males for suppression of horn flies

A RECENT trend in the control of insects has been the development of pest management systems whereby combinations of control methods, applied to one or more stages of the life cycle, increase efficiency and optimise effectiveness of individual methods. The potential of an integrated programme involving the release of sterile insects in combination with other control methods was discussed by Knipling[1]. He pointed out that two properly chosen and implemented systems might be regarded as synergistic in effect. Kunz and Eschle[2] have proposed an integrated programme against the horn fly, *Haemotobia irritans* (L.), and they have suppressed the reproduction of a semi-isolated population 98% by releasing sterilised horn flies[3]. In the spring of 1973, we began a pilot test on the island of Molokai, Hawaii. The objective was to develop a control system whereby a natural population of horn flies could be suppressed by chemical means and then eradicated through the release of sterile flies.

Harris *et al.*[4] have demonstrated that the development of horn flies is inhibited in the faeces of cattle fed methoprene (isopropyl (*E*,*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), an insect growth regulator (IGR). Because methoprene would affect only the reproduction of the native population, it could be used simultaneously with releases of sterile insects. In a preliminary experiment on the Kalaupapa peninsula of Molokai, continuous treatment of the drinking water of cattle with methoprene for 6 weeks reduced the estimated number of horn flies from 360 to seven per animal[5].

We report here the elimination of a wild population of horn flies through the simultaneous application of the IGR and the release of sterile males. The experiment was begun March 1975 on Puu-O-Hoku Ranch (POH), a 3,000-acre ranch with improved pasture and about 900 cattle (cows and calves), primarily Charolais and some Hereford.

POH is at the extreme eastern end of Molokai. The north-easterly trade wind, prevalent throughout the year in Hawaii, has a mean flow of 20 knots at POH and through the Pailolo channel that separates Molokai from Maui; it blows an average 90% of the time during April to September[6]. Theoretically, the test site was thus afforded some degree of protection from reinfestation by horn flies moving from cattle on other parts of Molokai and also on the island of Maui 9 miles to the south-east. When we released about 1.5×10^6 marked horn flies on the west end of Maui, none were recovered from cattle on Molokai. The only movement of marked flies observed when releases were made on Molokai was from east to west and from north-east to south-west.

The experiment was conducted on the north-eastern section of POH only (about 750 cattle) so that all other cattle on Molokai were downwind from the test area. South-west POH was not included because the pastures there contained streams which were impractical to treat with the IGR.

Methoprene was added to the drinking water of the test cattle as a tablet formulation. An automatic dispensing system[7] was attached to the outlet of the float-controlled valves of each water trough. Thus, the water was maintained with a concentration of 0.05–0.1 p.p.m. of methoprene, a level in the drinking water adequate to inhibit development of the horn fly in the manure of treated cattle[7]. Levels of methoprene in water samples from selected troughs were determined by gas chromatography. Effectiveness of treatments was confirmed by a bioassay in which samples of field-collected manure were seeded with horn fly eggs.

The sterile horn flies were provided from two colonies, one at Kerrville and another in Hawaii on the island of Oahu[8]. Chilled adult flies were sterilised with 2,500 rad (^{137}Cs source in Kerrville and ^{60}Co in Hawaii),

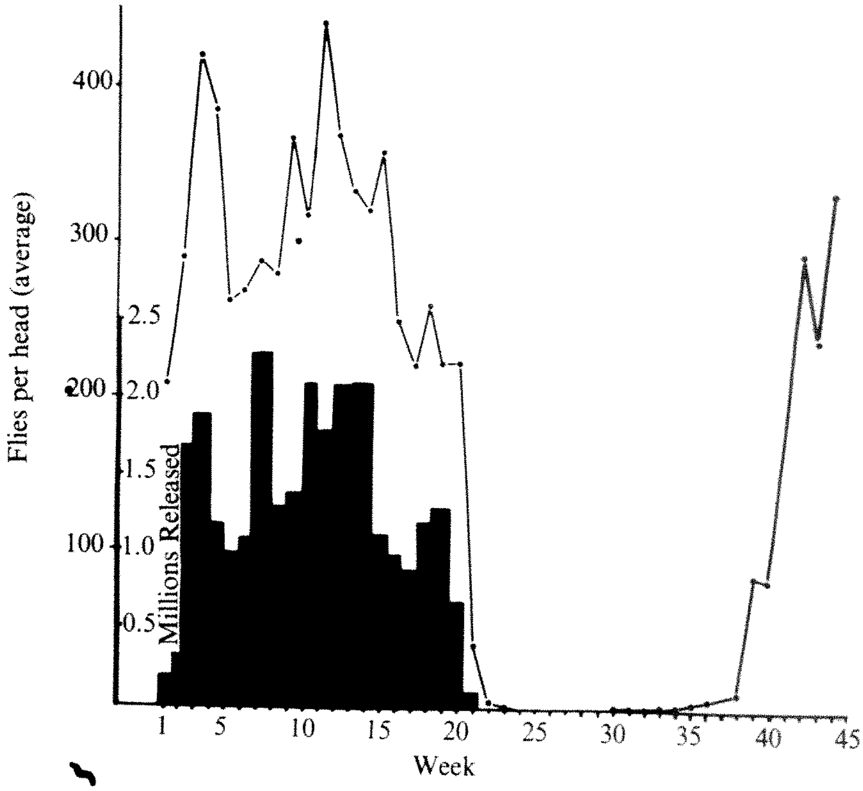


Fig. 1 Numbers of sterile horn flies released per week (bar graph) and estimated numbers of adult horn flies per head of cattle (line graph) on Puu-O-Hoku Ranch (March 17, 1975 to January 18, 1976).

marked with a fluorescent pigment, and placed in perforated paper bags. The bags were sealed in insulated containers and shipped by air to Molokai (in transit about 30 h from Kerrville and 16 h from Oahu). During shipment the flies were maintained in a chilled state by frozen eutectic material; O_2 was provided to sustain the lower metabolism, and an absorbent was used to avoid accumulation of CO_2.

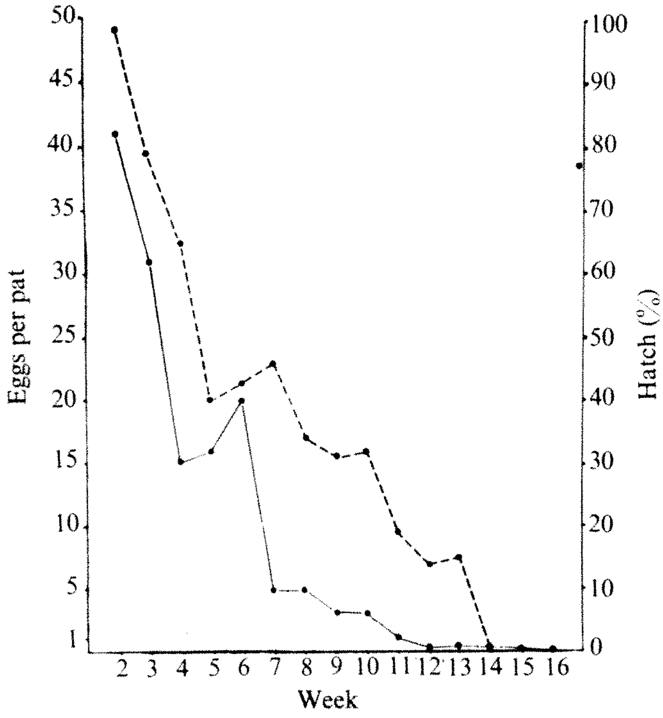
Sterile flies were released daily in the vicinity of cattle. Although released flies were marked, we could not consistently obtain samples of adult flies from test cattle to determine the ratio of released to native flies. Therefore, the frequency and rate of release in each pasture were determined by the number of flies available, the need for sterile flies as indicated by the current sterility of eggs sampled from the pasture, and the estimated number of adult horn flies on the cattle. From the volume (ml) of dead flies that remained 24 h after release, we estimated that more than 80% of the sterilised flies escaped from the release boxes.

The combined effect of the IGR and release of sterile flies were assessed from estimates of numbers of adult horn flies per animal and from numbers and fertility of horn fly eggs collected from manure pats in test pastures[3].

Methoprene treatment began on March 13–14, and release of sterile flies 1 week later (week 1). For the first 14 weeks of the experiment an average of 1.6×10^6 sterile flies per week were released. The population of adult flies (Fig. 1) averaged 300 per animal for weeks 1 to 14. But a steady decline in numbers and fertility of eggs, from an average 41 eggs per pat and 98% hatch during week 2 to 0.4 eggs per pat and complete sterility during week 14, indicated a decline in the population of native horn flies (Fig. 2). Therefore, we reduced the number of sterile flies released by about half (an average of slightly more than 800,000 per week). Beginning on week 16, no horn fly eggs were found on any of the pats in spite of intensified examinations; this indicated that the native population had been eliminated.

Release of sterile flies in the test pastures was discontinued after week 21. To prevent reinfestation, however, we released the bulk of sterile flies on cattle in south-west POH and made periodic, low-level releases in a buffer zone (pastures on the south-west border of the test area). Three weeks after the last release and for the next 6 weeks no adult horn flies were found on the cattle nor eggs in the manure. Meanwhile we continued to find a few, mostly sterile, eggs in the buffer zone, an indication that releases there were effective. Nevertheless, reinfestation occurred during week 30 when cattle from south-west POH were moved into the test area without our knowledge. By week 39, native populations had become re-established.

Fig. 2 Effect of combined use of methoprene and the sterile-male technique on production and fertility of eggs from horn flies on Puu-O-Hoku Ranch (March 24 to July 6, 1975). ——, Eggs per pat; – – –, per cent hatched.



All treatments were discontinued on POH during week 39. At that time we were devoting more attention to treatments in the buffer zone, and on south-west POH and other ports of Molokai. Thus we were unable to maintain the methoprene treatments as carefully as during the initial weeks of the experiment. The concentration of methoprene in water samples was found to be inadequate (<0.05 p.p.m.) in five of six troughs sampled in the test area during week 39. The population of adult horn flies had increased to an average 333 per animal by week 44 (Fig. 1).

The experiment demonstrated that methoprene in combination with releases of sterile males can be used to eliminate a native population of horn flies. Furthermore, the results suggested a strategy that could be used to implement a suppression–eradication programme involving a larger area such as the entire island of Molokai and, ultimately, the entire state of Hawaii. The control system could be used in a stepwise sweep from one end of the island to the other; the sweep would be planned so as to take advantage of natural isolating factors such as wind and geographical characteristics. A major advantage of such a strategy is that the size of the control area in each step could be controlled conveniently to fit the available personnel and mass rearing capabilities.

Implementation of the system on a large scale would require further refinements in rearing methods, the registration of methoprene for use in drinking water of cattle, and an understanding of the economic benefits.

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Y chromosome controls mating behaviour on *Anopheles* mosquitoes

SEVERAL species of *Culex* and *Anopheles* mosquitoes differ in their ability to breed in a relatively small space under laboratory conditions. When paired matings or mass rearings are carried out in small laboratory cages, males of some species are able to copulate while those of others are not. These characteristics have been named respectively stenogamy and eurygamy and reflect the field requirement for a small or a large space to perform the pre-copulative

Table 1 Results of paired matings between *Anopheles atroparvus* × *Anopheles labranchiae* hybrids with different sex chromosome combinations

Cross no.	Parents ♂	♀	Offspring code	Sex chromosomes in the male parent	Stenogamy in the male parent	Total	Paired matings No. fertile	No. sterile
1	*A. a.*	*A. a.*	*A. a.*	X_aY_a	yes	10	10	0
2*	*A. l.*	*A. l.*	*A. l.*	X_lY_l	no	10	10	0
3	*A. a.*	*A. l.*	I	X_aY_a	yes	19	19	0
4‡	I	I	II	X_lY_a	yes	9	1	8
5§	I	*A. a.*	III	X_lY_a	yes	—	—	—
6	*A. a.*	I	IV	X_aY_a	yes	11	11	0
7¶	IV	IV	V	X_aY_a; X_lY_a	yes	10	6	4
8	IV	*A. a.*	VI	X_aY_a; X_lY_a	yes	13	6	7
9	*A. a.*	IV	VII	X_aY_a	yes	9	9	0
10*	*A. l.*	I	VIII	X_lY_l	no	7	7	0
11§	VIII	*A. a.*	IX	X_aY_l; X_lY_l	no	15	6	9
12§	VIII	VIII	X	X_aY_l; X_lY_l	no	7	4	3
13	*A. a.*	VIII	XI	X_aY_a	yes	10	10	0
14§	*A. l.*	*A. a.*	XII	X_lY_l	no	6	6	0
15§	XII	XII	XIII	X_aY_l	no	2	0	2
16	*A. a.*	XII	XIV	X_aY_a	yes	12	12	0
17	XIV	XIV	XV	X_lY_a; X_aY_a	yes	8	4	4
18§	*A. l.*	XII	XVI	X_lY_l	no	5	5	0
19*	XVI	XVI	XVII	X_lY_l; X_aY_l	no	11	4	7

A. a. = *Anopheles atroparvus*; *A. l.* = *Anopheles labranchiae*. The subscripts *a* and *l* under X and Y indicate their origin from *A. a.* and *A. l.* respectively. The *A. a.* strain has been reared in the laboratory for 20 yr. The *A. l.* mosquitoes were captured in the North of Sardinia as 3rd or 4th instar larvae. All crosses were performed in 20-cm cubic cages kept at room temperature of 25–28 °C, with humidity of 80–90%, in an undarkened room. Crosses were made by paired mating, using males at least 5 d old and females to which one or two blood meals had been given. Mass rearing was performed in the same type of cages with the number of mosquitoes given in parentheses: cross 5 (58 ♂ × 21 ♀); cross 7 (80 ♂ × 40 ♀); cross 11 (7 ♂ × 5♀); cross 12 (50 ♂ × 25 ♀); cross 14 (257 ♂ × 126 ♀); cross 15 (23 ♂ × 18 ♀); cross 18 (5 ♂ × 3 ♀).

*Artificial insemination.

‡ Only one female offspring was reared from this cross.

§Mass rearing resulted sterile.

¶Mass rearing resulted fertile.

flight[1]: in stenogamous species the male performs an almost stationary flight with short, quick movements followed by mating with the female which remains motionless on a wall; conversely, the males of eurigamous species form small swarms of several individuals into or near which the females fly and copulation takes place in flight. These behavioural characteristics are inherited and, although the dominance of stenogamy over eurygamy has been suggested[2–5], previous data are inconclusive mainly because of the lack of informative F_2 and backcross experiments[5]. In fact, in *Anopheles*, though all types of F_1 hybrid females are fertile when backcrossed to the parental species, the F_1 hybrid males are usually sterile[5].

To investigate the genetic control of the eurygamy/stenogamy polymorphism we did a series of crosses between the sibling species of the genus *Anopheles*, *A. labranchiae*, which is eurygamous and *A. atroparvus* which is stenogamous, using artificial insemination[6] whenever the male was unable to copulate because of its stenogamous behaviour. For comparison we also performed a number of mass rearings. We found that the polymorphism is controlled by the Y chromosome

The results of our crosses are shown in Table 1, in which the basic character taken into consideration is stenogamy in the male parent. These experiments first confirmed the sterility of all males which are hybrids for the sex chromosomes irrespective of their autosomal constitution; this is obvious from crosses 4, 5 and 15. Whenever the sex chromosomes belong to the same species, the male is fertile, as seen from crosses 6, 9, 10, 13. 16 and 18. Furthermore, when segregation for the X chromosomes of the two species is expected in the male progeny and these males are crossed, a corresponding 1 : 1 ratio of sterile and fertile paired matings is found, as in crosses 7, 8, 11, 12, 17 and 19. We made histological and meiotic studies of the sterile males and found that the sterility is due to arrest of spermatogenesis before first meiotic metaphase.

Whenever the male carries the Y chromosome of *A. atroparvus* the mating behaviour is stenogamous and copulation takes place spontaneously in cages, as shown by crosses 1, 3, 4, 5, 6, 7, 8, 9, 13, 16 and 17. Conversely, all males with the Y chromosome of *A. labranchiae* are eurygamous (see crosses 2, 10, 11, 12, 14, 15, 18 and 19) and artificial insemination was necessary for their reproduction.

Reproductive success of the male is therefore controlled on the one hand by the Y chromosome which determines mating behaviour and on the other by testicular fertility which is determined by the constitution of the XY pair, as exemplified in Table 2.

Table 2 Sex chromosome constitution controls testicular fertility and pre-copulative flight behaviour in *A. atroparvus* (*a*) × *A. labranchiae* (*l*) *hybrids*

	Testicular sterility	Testicular fertility
Eurygamy	X_aY_l	X_lY_l
Stenogamy	X_lY_a	X_aY_a

In conclusion, the behavioural polymorphism stenogamy–eurygamy is obviously controlled by one or more genes located on the Y chromosome. To our knowledge this is the first example of direct chromosomal control of a behavioural characteristic involved in reproduction.

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Cadmium tolerance in populations of *Agrostis tenuis* and *Festuca ovina*

IT is well known that plant populations growing on soils contaminated with heavy metals are genetically tolerant to those metals. This adaptation is not found in populations growing on normal soils (ref. 1 and review in ref. 2). Most of the literature is concerned with the resistance of plants to Pb, Zn and Cu[3–8], but Cd salts too are often present in the soils of areas polluted by those metals. I have therefore investigated the occurence of Cd tolerance in some Belgian and German populations of *Agrostis tenuis* Sibth. and *Festuca ovina* L. *sensu lato*.

Population samples of at least 25 individuals of *A. tenuis* and *F. ovina* were collected from sites contaminated with heavy metals and from normal sites, and transplanted for 2 yr in an experimental garden. Populations are described in Table 1. Soil samples corresponding to each population were analysed in three ways. (1) Total heavy metals were measured by extraction with concentrated nitric acid at 25 °C (10-g soil sample, dried at 105 °C with 25 ml of HNO_3 for 48 h). (2) Exchangeable cations were measured by extraction with 1 M NH_4COOH at *p*H 7. (3) Water-soluble elements were measured by extraction in distilled water at 25 °C (10-g soil sample, dried at 105 °C, shaken, in 25 ml of H_2O for 48 h). Plant samples (aerial green parts) collected at random in the different localities were dried at 105 °C and reduced to powder. Samples of 1 g of powder were digested with a mixture of nitric and perchloric acids. After filtration the samples were analysed by atomic absorption for Cd, Zn and Pb.

Table 1 Localities and plant populations with their characteristics

Localities	Description of localities	Species collected and transplanted
Belgium, Plombières	Old Pb and Zn mine in eastern part of Province de Liège; soil very rich in Pb and Zn in form of ore and blast furnace scorias; vegetation consists of turf of *F. ovina*, *Armeria maritima*, *Viola calaminaria*; soil *p*H 6.0–6.5	*Festuca ovina* and *Agrostis tenuis*
Belgium, Prayon	Industrial area near Liège characterised by aerial pollution consisting of Zn, Pb, Cd and SO_2; old soils colonised only by *A. tenuis* and *Thlaspi coerulescens* ssp. *calaminare*; soil *p*H 4.5–5.5	*Agrostis tenuis*
Belgium, Trazegnies	Uncontaminated area near Charleroi; soil *p*H 6.0–6.5	*Agrostis tenuis*
Belgium, Frasneslez-Couvin	Uncontaminated area in Province de Namur; turf with *F. ovina* and *Bromus erectus*; calcareous soil	*Festuca ovina*
Germany, Breinig	Near Aachen; calcareous hills with Zn and Pb ore; turf rich in *F. ovina*, *Bromus erectus*, *Armeria maritima*, *Viola calaminaria*, etc.	*Festuca ovina*
Germany, Mausbach	Near Aachen; sandy artificial hill formed by accumulation of the tailings of Zn and Pb ores; vegetation includes *Molinia coerulea*, *F. ovina* and *Armeria maritima*	*Festuca ovina*
Germany, Strempt	Near Aachen; sandy artificial wastes formed by accumulation of tailings of Zn and Pb ores; vegetation includes mainly *F. ovina* and *A. tenuis*	*Festuca ovina*

Table 2 Amounts of Cd, Zn and Pb in the contaminated soils of Plombières, Breinig, Mausbach, Strempt and Prayon

Site	*p*H	Organic matter %	Nitric acid extraction			Ammonium acetate extraction			Distilled water extraction		
			Cd	Zn	Pb	Cd	Zn	Pb	Cd	Zn	Pb
Plombières, from a well developed turf	6.3	17.2	31.0	29,087	9,126	14.3	3,327	1,682	1.0	54.8	0.8
Plombières, turf at an early stage of development	6.1	10.5	69.8	20,616	26,140	17.7	1,337	4,515	1.0	19.0	1.0
Breinig, from a well developed turf	7.5	14.0	144.0	41,275	10,269	27.3	1,631	568	0.0	7.6	—
Mausbach, from a turf at an early stage of development	7.2	9.5	59.1	14,850	6,675	13.8	927	494	0.0	5.6	—
Strempt, from a turf at an early stage of development	5.6	9.4	2.0	2,250	18,000	0.0	230	1,293	0.0	16.5	0.5
Prayon, well developed turf	6.3	—	135.0	5,300	1,100	19.5	427	70	0.0	—	—

Figures are given in mg per kg of dried soil at 105 °C; means of 20 samples.

Heavy metal tolerance was determined by Wilkins's method, based on comparative root growth in toxic and non-toxic solutions. In each population 25 individual tillers were rooted in distilled water and the longest root of each was measured. The rooted tillers were then transferred to heavy metal solutions at different concentrations; a test sample was maintained in distilled water. After 5 d the longest root was measured again. The ratio

$$100\times\frac{\text{length of the longest root in toxic solution}}{\text{length of the longest root in non-toxic solution}}$$

is known as 'the tolerance index' of the population for a given metal at a specific concentration. The same procedure was used to study the effects of soil nutrients (Ca, Mg, K and Na added as sulphate or nitrate) on the tolerance level of plant populations.

In the samples from normal areas, we found small amounts of total Cd (1.0 mg kg^{-1} from Frasnes and 0.85 mg kg^{-1} from Trazegnies). No exchangeable or soluble Cd was found. In the contaminated soils (Table 2) Cd reached high levels, being greatest in soils from Breinig (calcareous), but the mobility of the metal was much higher in the acid soils of Plombières. Smaller amounts of Cd were found in soils from Prayon. Soils from Strempt (Germany) were rich in Zn and Pb but no significant quantity of Cd was found.

Ernst[10] considers that the amount of exchangeable metal is the maximum available to plants and that the soluble

Table 3 Amounts of Cd, Zn and Pb in plants from polluted areas

Localisation and species	Date of collection	Cd	Zn	Pb
Plombières, *A. tenuis*	5/74	1.0	372.0	129.0
Plombières, *F. ovina*	5/74	0.5	390.0	175.0
Breinig, *F. ovina*	6/74	1.6	179.0	43.0
Breinig, *Euphrasia stricta*	6/74	11.0	937.0	42.0
Breinig, *Hieracium pilosella*	6/74	22.0	483.0	34.0

Figures are given in mg per kg of dry weight.

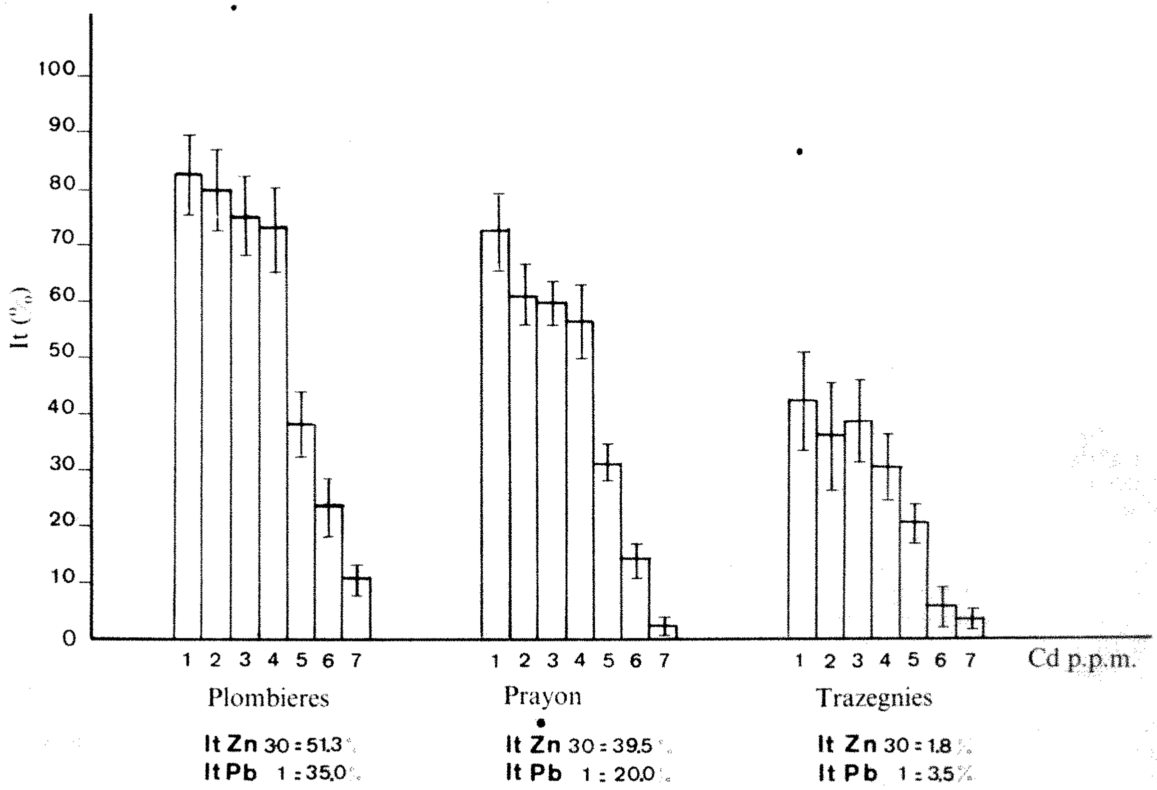

Fig. 1 Cd tolerance in *A. tenuis* populations at different concentrations of Cd. Plombières and Prayon are the contaminated areas. Trazegnies is the test population. Tolerance indices are given as percentages with standard errors. 1–7, Cd concentrations in the culture solutions: 1, 0.25 p.p.m.; 2, 0.50 p.p.m.; 3, 0.75 p.p.m.; 4, 1.00 p.p.m.; 5, 1.50 p.p.m.; 6, 2.00 p.p.m.; 7, 2.50 p.p.m. It Zn 30 is the tolerance index to Zn for the population grown on a solution with 30 p.p.m. of Zn. It Pb 1 is the tolerance index to Pb for the population grown on a solution with 1 p.p.m. of Pb.

fraction is the minimum available. Amounts of soluble Cd were very low compared with Zn (Table 2). My results for *A. tenuis, F. ovina* and some dicotyledons from Plombières and Breinig are shown in Table 3, together with values for Zn and Pb. Cd values were higher for dicotyledons than for monocotyledons. *F. ovina* from Breinig (calcareous soil) contained more Cd than did the population from Plombières. The opposite pattern was found for Zn and Pb. This relates to the differential mobility of metals in relation to edaphic conditions.

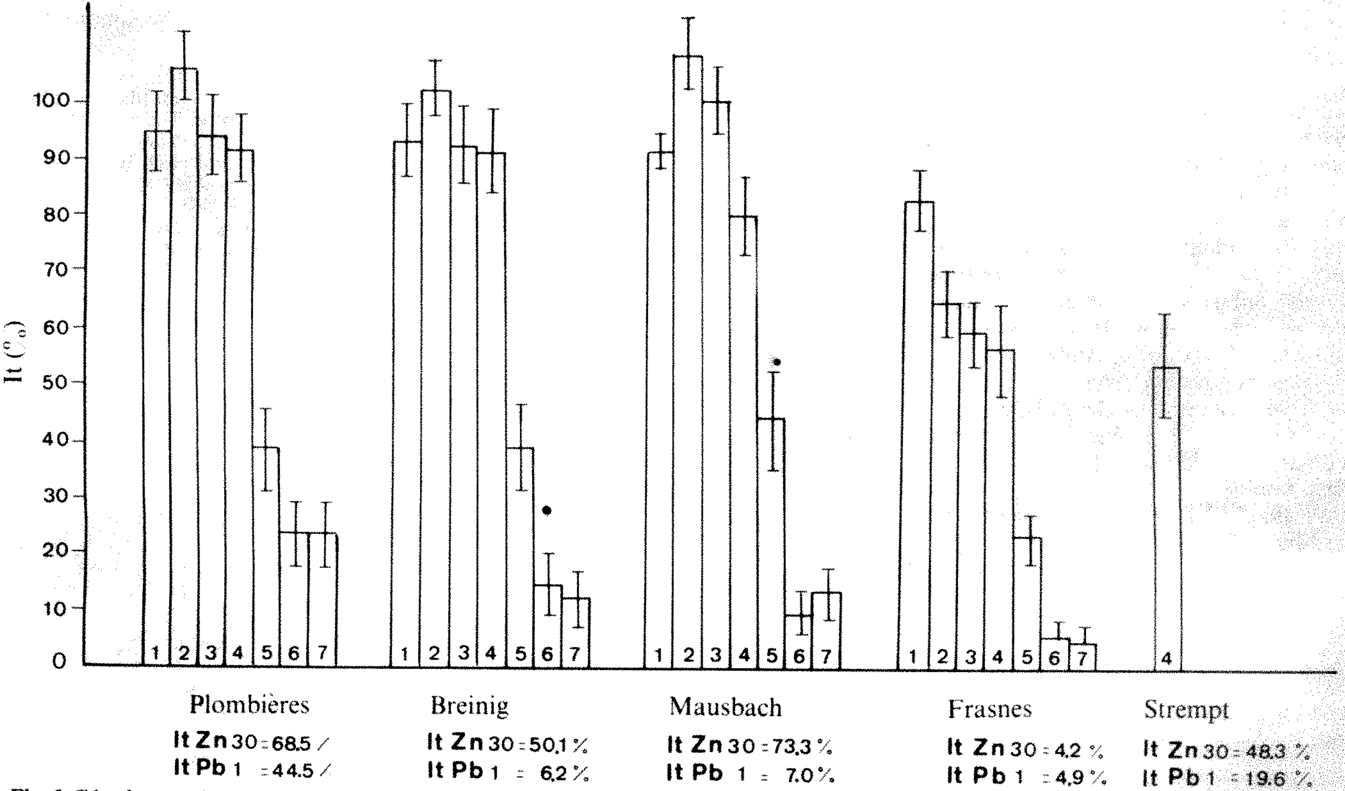

Fig. 2 Cd tolerance in populations of *F. ovina* at different concentrations of Cd. Plombières, Breinig and Mausbach are populations from contaminated areas. Frasnes and Strempt are the test population but Strempt is an area contaminated with Zn and Pb. The tolerance indices are given as percentages with standard errors. 1–7 are Cd concentrations in the culture solutions. 1, 0.25 p.p.m.; 2, 0.50 p.p.m.; 3, 0.75 p.p.m.; 4, 1.00 p.p.m.; 5, 1.50 p.p.m.; 6, 2.00 p.p.m.; 7, 2.50 p.p.m. It Zn 30 is the tolerance index to Zn for the population grown on a solution with 30 p.p.m. of Zn. It Pb 1 is the tolerance index to Pb for the population grown on a solution with 1 p.p.m. of Pb.

Table 4 Effect of some soil nutrients on the toxicity of Cd

Species	Populations	Cd 1 p.p.m.	Cd 1 p.p.m. +Ca 5×10⁻⁴ M	Cd 1 p.p.m. +Mg 5×10⁻⁴ M	Cd 1 p.p.m. +K 5×10⁻⁴ M	Cd 1 p.p.m. +Na 5×10⁻⁴ M
	Plombières	83.2 ± 6.9	97.8 ± 6.3	84.1 ± 6.9	118.6 ± 9.5	85.2 ± 6.6
A. tenuis	Prayon	62.3 ± 6.0	99.2 ± 6.2	70.6 ± 6.2	85.3 ± 9.6	58.2 ± 4.3
	Trazegnies	41.4 ± 6.1	106.6 ± 9.0	92.8 ± 9.2	96.3 ± 7.3	70.9 ± 6.3
	Plombières	90.4 ± 5.3	96.5 ± 6.4	89.5 ± 6.3	99.4 ± 7.0	86.4 ± 6.0
F. ovina	Breinig	89.7 ± 6.0	98.4 ± 6.0	91.4 ± 6.5	88.6 ± 6.8	87.5 ± 5.5
	Frasnes	56.5 ± 6.0	85.5 ± 5.7	89.7 ± 8.0	88.0 ± 6.7	60.2 ± 5.0

Tolerance indices are reported in % with standard errors. Plombières, Prayon and Breinig are the contaminated areas. Trazegnies and Frasnes-lez-Couvin are normal areas.

I next tested for Cd tolerance in populations of *A. tenuis* and *F. ovina* from the contaminated areas of Plombières, Prayon, Breinig, Mausbach and Strempt. As Figs 1 and 2 show, the populations of the contaminated areas were more tolerant than those from normal areas. Tolerance indices were very high for *A. tenuis* from Plombières and Prayon, but much lower for this species from the normal soil of Trazegnies. *A. Tenuis* populations from Plombières and Prayon are therefore genetically adapted to Cd toxicity. With *F. ovina*, high tolerance indices were found for the populations from Plombières, Breinig and Mausbach compared, with those for the population from the normal soil of Frasnes.

Thus my work on transplanted populations shows that Cd tolerance has a genetic basis. The population of Strempt was not tolerant to Cd even though it was tolerant to Zn and Pb (Fig. 2). In *A. tenuis* and *F. ovina* populations, however, a decrease in the tolerance index was generally observed at 1.50 p.p.m. Cd. At 2.50 p.p.m. (except the population from Plombières) the plants stopped growing. In addition, in Plombières *A. tenuis* was always less tolerant than *F. ovina* to the three heavy metals tested.

The effects of some soil nutrients were studied. *A. tenuis* and *F. ovina* populations from contaminated and normal soils were grown in a solution containing Cd $(NO_3)_2$ (1 p.p.m.) and different soil nutrients $Ca(NO_3)_2$, $MgSO_4$, K_2SO_4 and Na_2SO_4 each at 5×10^{-4} M. Table 4 shows that the addition of Ca, Mg or K to the Cd solution restored normal growth to the populations of both species, even when they had been collected from normal soils. Na did not reduce the toxicity of Cd. This effect has been observed for the same populations grown on Zn and Pb solutions[8].

Cd has high mobility and is available to plants. In acidic soils solubilisation makes this availability greater than in basic soils. Plants from soils rich in Cd are genetically more tolerant to Cd than plants from normal soils. The significant differences in tolerance indexes between *F. ovira* and *A. tenuis* show that tolerance to Cd is a specific character. Apparently, cotolerance does not occur, for the *F. ovina* population from Strempt was not tolerant to Cd but it was tolerant to Zn and Pb.

Cd tolerance is probably not restricted to a few species of Poaceae and it would be interesting to test species from other families.

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Self-thinning driven by leaf area not by weight

THE self-thinning rule is perhaps the most widely applicable principle in plant population dynamics. White[1] has shown that it applies to more than 70 species ranging from herbs to trees, in many nutrient conditions. The rule states that $W=Kp^{-3/2}$, where W is average plant weight, p is the density of plants, and K is a constant. In other words when the log of average weight is plotted against the log of average density for a crowded even-aged plant population, numbers must be lost as the individuals gain weight in such a way that the population's trajectory is held under a line of slope $-3/2$.

Why the slope should be $-3/2$ has been unclear. The dimensional explanation has been put forward[2] that the weight of a plant increases as the cube of a size dimension, while the area occupied by it increases as the square of a

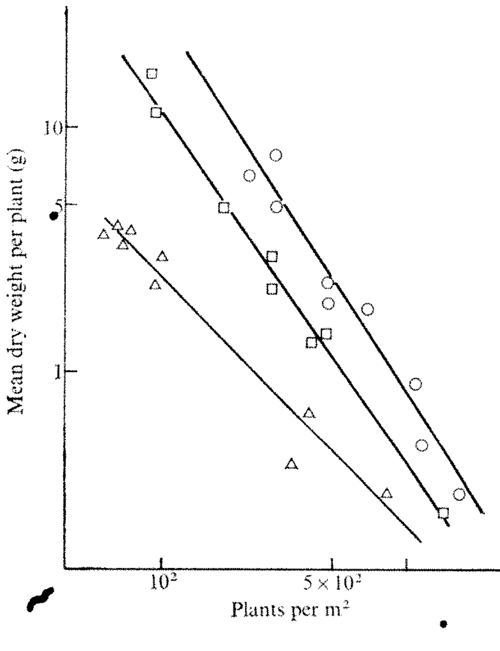

Fig. 1 Changes in density (p) and mean individual plant weight (W) of *Helianthus annuus* populations with time. The populations were grown at 100% (○), 60% (□) and 23% (△) light intensity. Data from starting densities of 1,600 and 400 plants per m^{-2} are combined. Thinning lines shown are for 100% light log $W = 3.84–1.33 \log p$, for 60% light $\log W = 3.53-1.30 \log p$, and for 23% light $\log W = 2.50-1.08 \log p$. After White and Harper[2], from data of Hiroi and Monsi[4].

size dimension. But this reasoning does not explain the only significant exception to the −3/2 slope which has been found, which is that the slope is flatter under deep shade[2,3]. This contribution shows that plants in low light develop more leaf area per unit weight, and that populations in both low and high light thin along the same slope in relation to leaf area.

I have found data where light levels were varied in studies both on self-thinning, and on the relation of leaf area to plant weight, only for *Helianthus annuus*. Self-thinning was studied by Hiroi and Monsi[4] and analysed by White and Harper[2]. The flattening of the slope of self-thinning at low light intensity is shown in Fig. 1. Blackman and Wilson[5] documented leaf area–plant weight relationships in different light intensities. Figure 2 shows that while leaf area per unit plant weight is constant at any one light level, it is greater in shade than in full light.

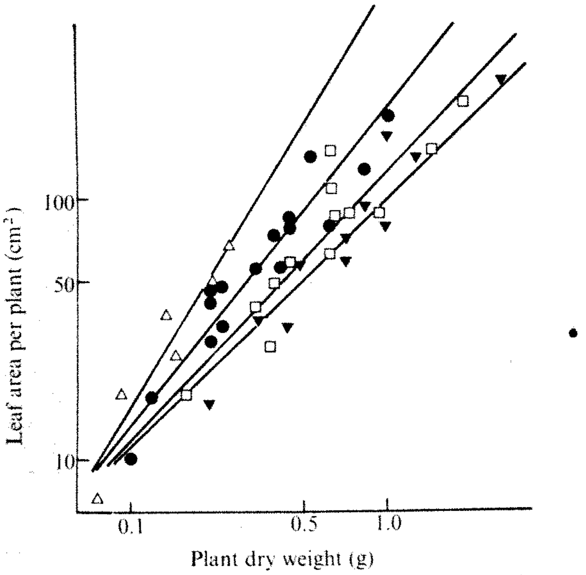

Fig. 2 Relation of leaf area per plant in cm² (L) to individual plant weight in g (W) at different light levels for *Helianthus annuus*, from data of Blackman and Wilson[5]. Data for six experiments are combined. Light intensities were 100% (▼), 50% (□), 24% (●) and 12% (△) of full daylight. Best fit equations are, for 100% light, $\log L = 2.01 + 0.98 \log W$, for 50% light $\log L = 2.11 + 1.05 \log W$, for 24% light $\log L = 2.36 + 1.24 \log W$, and for 12% light $\log L = 2.83 + 1.63 \log W$. All equations have r^2 greater than 0.9. Where there were identical data points only one, from the lowest light level, is shown here, but all were used in calculating equations.

Both the flatter thinning slope, and the higher leaf area per unit weight, under low light, are common to all plant species studied[3,5,6], though *H. annuus* seems to be the only species for which both are documented.

The equations of Fig. 2 can be used to transform the data of Fig. 1 from a plot of weight against numbers to a plot of leaf area against numbers (Fig. 3). This completely removes the anomalous behaviour of the system at low light intensities. Thus it seems that the self-thinning rule in its most general form is $L = Kp^{-3/2}$.

Now the behaviour of plant populations while self-thinning is characterised (1) by the −3/2 slope just shown to be general if leaf area rather than weight is used as abscissa, (2) by the constant K, and (3) by the speed with which the population moves along the trajectory defined by $L = Kp^{-3/2}$, and the place where it stops. Formulating the self-thinning rule in terms of leaf area allows some further deductions on the third of these points. Since Lp is equal to the leaf area index (LAI) or area of leaf per unit ground area we can write $\mathrm{LAI} = Kp^{-1/2}$. Thus we can relate the relative mortality rate $(-(\mathrm{d}p/\mathrm{d}t)/p)$ of plants to the relative growth rate of the LAI $((\mathrm{dLAI}/\mathrm{d}t)/\mathrm{LAI})$; specifically $(-(\mathrm{d}p/\mathrm{d}t)/p) = 2((\mathrm{dLAI}/\mathrm{d}t)/\mathrm{LAI})$. Thus the time dynamics of mortality are driven by the function relating dLAI/dt to LAI, a relationship which has been studied for several crops. In particular, we expect no further thinning when the canopy reaches maximum LAI[6].

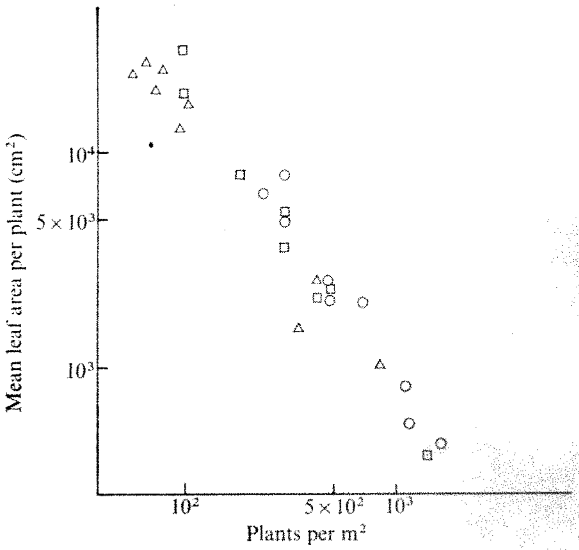

Fig. 3 Data on self-thinning in *Helianthus annuus* of Fig. 1 with average weight per plant transformed into average leaf area per plant using the equations appropriate to each light level from Fig. 2. Symbols for light intensities as in Fig. 1.

It is therefore to be hoped that future work on self-thinning will include measurements of leaf area as well as of weight and numbers of plants. As White and Harper[2] said when formulating the self-thinning rule: "It would be of great theoretical interest and of no little practical significance to determine the parameters of K for even a single species". Perhaps it will prove possible to explain K in terms of those canopy properties which determine optimal and maximum leaf area indices[6].

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New structure associated with stomatal complex of the fern *Polypodium vulgare*

THE opening movements of stomata are generally considered to result from the guard cells gaining an osmotic advantage over their immediately adjacent epidermal cells resulting in the uptake of water into the guard cells and their consequent increase in volume. Fujino[1] and Fischer[2] independently proposed that active potassium transport between the guard and epidermal cells could be the mechanism by which the cationic fraction of the osmotic imbalance could be achieved. In recent years many investigators have produced corroborative evidence for this potassium shunt[3] using Macallum's histochemical stain[4]. Further support for the hypothesis has been obtained using electron probe microanalysis[5–8] and potassium-sensitive microelectrodes[9]. Despite these studies, no clear-cut picture has emerged concerning either the reservoirs of the ions at either end of the flux or the intercellular ionic pathway although the findings of Heller *et al.*[10] implicate the vacuole as the ion reservoir within the guard cells as do those of Penny and Bowling[9]. There is no current evidence of where or, indeed, whether, potassium is sequestered after it is shunted out of the guard cells during stomatal closure, but it has been suggested that the differentially thickened cell walls of the stomatal complex could serve as storage sites[11,12]. We have recently discovered some new morphological features in the stomatal complex of the common polypody, *Polypodium vulgare* L., which we believe represent the site of potassium accumulation in this species.

Using the basic Macallum stain[4] and viewing the stained epidermal strip from the side adjacent to the mesophyll under a Zeiss Photomicroscope II, distinct polar accumulations of the ion can be observed in the guard-cell complexes (Fig. 1*a*). The accumulations are commonly up to $25\ \mu m$ in diameter and can extend by as much as $5\ \mu m$ beyond the guard cell walls at the poles (Fig. 1*b*). Associated with the guard cells, and especially with the polar accumulations, are potassium-rich strands that we construe as hollow trabeculae which bridge the common anticlinal walls of the guard and subsidiary cells. The polar accumulations show a distinct bimodal staining pattern which we interpret as indicative of (1) a darker, central circular site of ion adsorption and (2) a paler, bi-lobed osmotically/imbibitionally-induced swelling. This bimodal pattern is ill-defined in many guard-cell complexes because of the dense nature of the underlying chloroplasts but, interestingly, both elements of the polar structures are present in many dead guard cells which show no trace of protoplasts (Fig. 1*c*). Light microscopy studies suggest that the ion-adsorbent sites rest immediately on the outer surface of the guard-cells walls and that the osmotically/imbibitionally-induced sacs are located between the guard-cell walls and the endocuticle (Fig. 2).

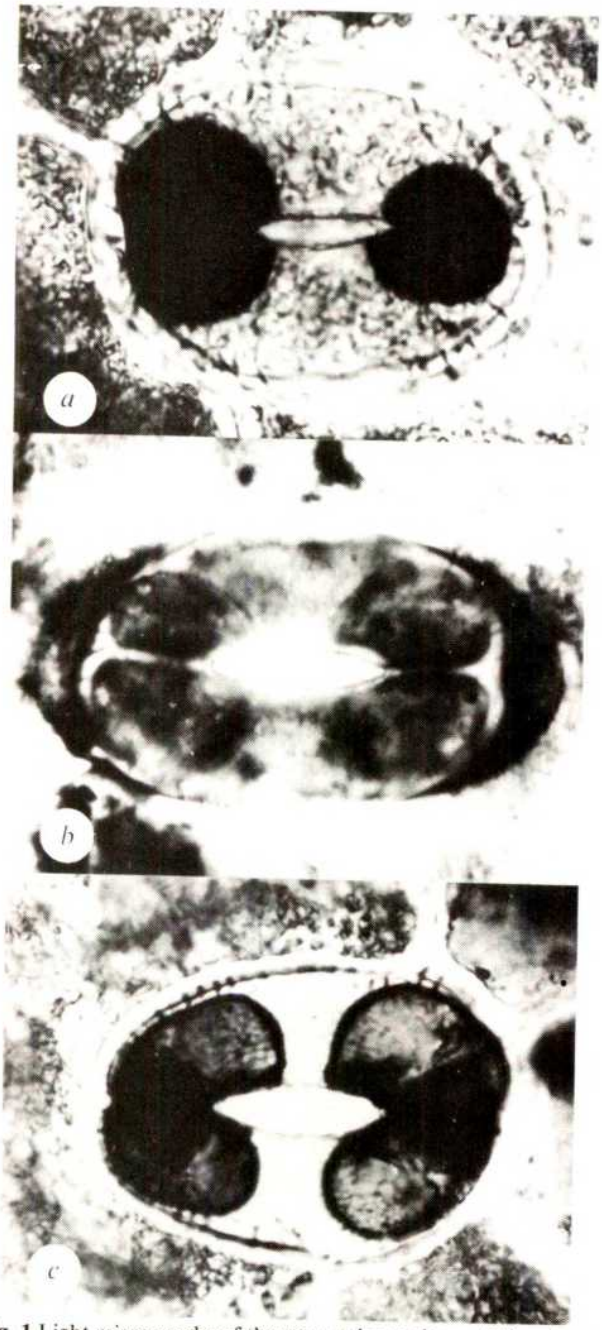


Fig. 1 Light micrographs of the stomatal complexes of *P. vulgare* stained for potassium using the Macallum technique, × 1,130. *a*, Living complex viewed from the side adjacent to the mesophyll demonstrating the polar accumulation of the ion and the ion-rich trabeculae; *b*, living complex viewed from the outer surface demonstrating the polar accumulations subtending and extending beyond the polar cell walls of the guard cell complex; *c*, dead complex viewed from the side adjacent to the mesophyll demonstrating the two elements of the polar structures.

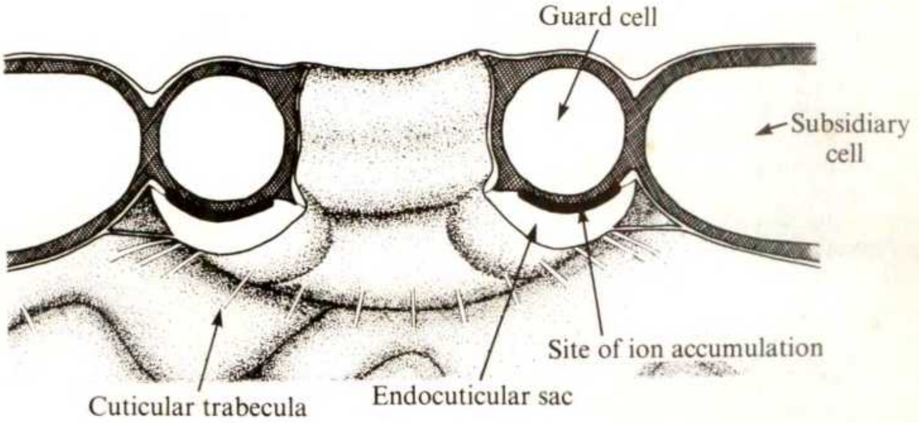
Fresh material examined in an inverted position in the cryo unit of JSM-35 scanning electron microscope clearly shows that the polar accumulations observed with the histochemical stain are compatible with discrete substomatal sacs at the poles of the guard-cell complex and that the trabeculae are formed from extensions of the endocuticle immediately above the common sutures of the guard and subsidiary cells (Fig. 3*a*). The substomatal cuticular swellings are considered to represent the osmotically/imbibitionally-inflated products of the underlying ion-adsorbent sites determined with the histochemical stain. Epidermal strips fixed in 70% ethanol and observed with the scanning electron microscope indicate the collapse of the endocuticle on to the underlying cell walls and what we believe to be the actual ion-adsorbent areas underneath the substomatal sacs (Fig. 3*b*).

Using similar techniques, we have established the presence of substomatal sacs and ion-adsorbent areas in a wide range of filicopsid species but their absence in the psilopsid, lycopsid and sphenopsid pteridophytes we have been able to examine. What seem to be identical structures have also been found in some members of the monocotyledon family Commelinaceae, namely *Tradescantia virginiana*, *T. pallidus*, *Commelina communis*, *Callisia fragrans* and *Palisota barteri* (Fig. 4).

We can find no previous report of substomatal sacs in the literature although 'polar vesicles' were reported in the guard cells of *Casuarina*[13] and analogous structures in the guard cells of *Psilotum*[14]. From these reports and our own studies on *Psilotum nudum*, it is clear that the polar vesicles are formed as a result of the dumb-bell-shaped lumen of the guard cells. Thickened common anticlinal guard-cell walls in *Vaccinium* spp. give rise to the appearance of polar pouches[15] but a re-examination of this structure in *Vaccinium myrtillus* leaves us in little doubt that they are artefacts caused by the subsidiary cells entirely subtending the guard cells except in the polar regions. Differentially thickened common guard cell walls have also been reported by Raju *et al.*[16]. We confirm the existence of these specialised regions and have found them in many of the species we have examined (including *Polypodium vulgare*) but do not believe that they are directly connected with the actual substomatal sacs we report here.

The functional significance of these new structures is difficult to determine at present owing to the subdued responses of fern stomata in general. We have been unable to produce any clear evidence that stomatal movements in *P. vulgare* are anything more than extremely limited in extent although Lange *et al.*[17] elicited responses to certain environmental conditions imposed on isolated epidermal

Fig. 2 Diagrammatic interpretation of a vertical longitudinal section through the polar structure in the guard cell complex of *P. vulgare*. The ion-adsorbent sites are represented by the solid black areas adjacent to the guard cell wall (cross-hatched) with the osmotically/imbibitionally swollen endocuticular sacs lying immediately below.



strips of this species. Meidner[12] records some inconsistencies between potassium content and stomatal aperture in the related fern, *Phyllitis scolopendrium*, a species which exhibits more typical stomatal responses[18]. We have obtained tentative evidence from scanning electron microscopy that the substomatal sacs become slightly smaller under dark conditions when compared with those under light conditions. Fern guard cells generally differ from those of typical angiosperms in having more numerous and larger chloroplasts which means that the vacuolar element of the protoplasts must be greatly reduced in volume. It could be that the substomatal sacs have been developed in *P. vulgare* and allied species to accommodate the accumulation of ions such as potassium in an extracellular position. If this is the case, it is difficult to see how the ions could have the necessary turgor effect on the guard cells required for stomatal movements. More probably, the function of the substomatal sacs is concerned with water conservation since cuticular water loss must be high in ferns because of the poor development of the cuticle in this group of plants. The inner faces of the guard and subsidiary cells are evaporative sites which make a major contribution to the water lost in

Fig. 3 Scanning electron micrographs of the stomatal complex of *P. vulgare* viewed from the side adjacent to the mesophyll, × 1,200. *a*, Fresh material showing the polar substomatal sacs and trabeculae. A secondary signal distribution along the stomatal pore is superimposed on the micrograph to give an idea of the surface topography in this region. *b*, Material preserved in 70% ethanol showing the ion-adsorbent areas at the poles of the guard-cell complex.

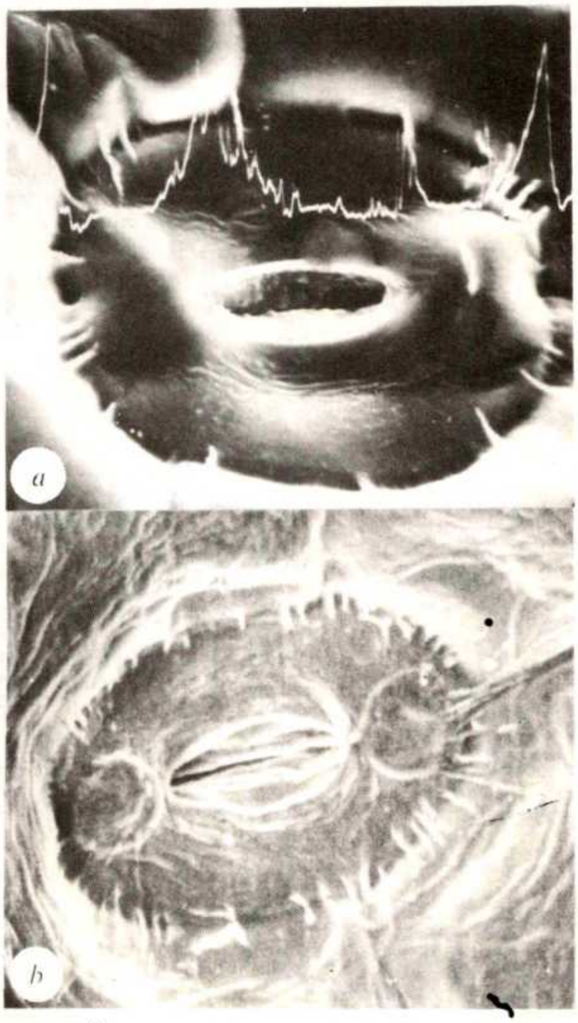
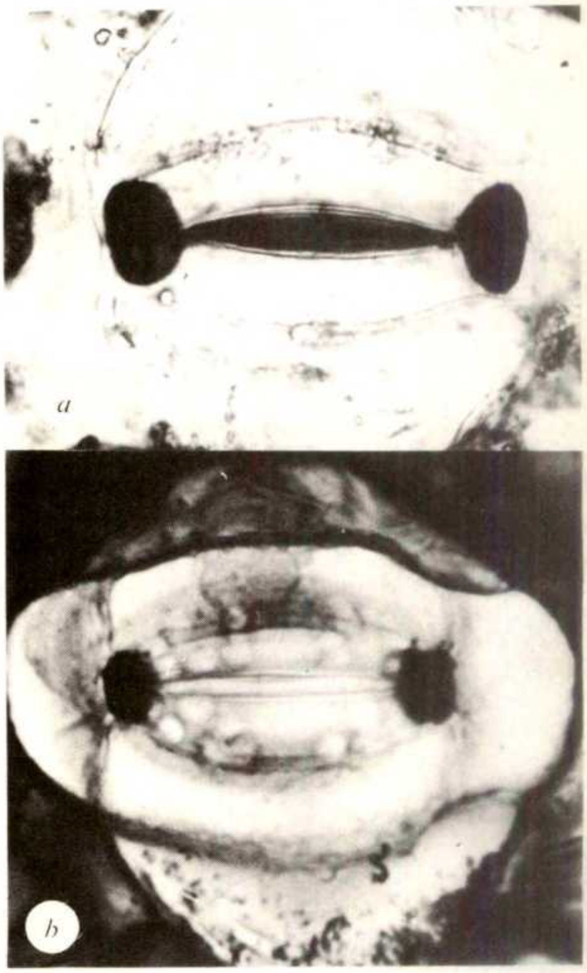


Fig. 4 Light micrographs of stomatal complexes viewed from the side adjacent to the mesophyll after staining for potassium using the Macallum technique. *a*, *Tradescantia virginiana*, × 790; *b*, *Commelina communis*, × 1,010.



stomatal transpiration[19]. The presence of potassium-rich sacs at these evaporative sites could reduce potential transpiration losses by virtue of their low osmotic potential and could also provide the guard-cell complex with a convenient source of cations for incorporation into the complex during stomatal opening.

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Stem anatomy and sheathing mycorrhizas in the *Betula verrucosa–Amanita muscaria* relationship

Increasingly, attempts are being made to afforest difficult sites including prairie and steppe lands, peat bogs, *Calluna* heaths and industrial wastelands. In the United Kingdom the Forestry Commission is planting Sitka spruce (*Picea sitchensis* (Bong. Carr.) and Lodgepole pine (*Pinus contorta* Loud.) on land subject to periodic or protracted waterlogging and, notwithstanding the use of improved techniques of planting and site preparation, these species tend to be shallow rooted and liable to 'wind blow'[1]. In these conditions the establishment of nursery-grown seedlings is problematical. As long ago as 1917, Melin[2] noted that while *Pinus sylvestris* L. and *Picea abies* (L.) Karst. transplants, used for afforesting freshly drained peat bogs, were without mycorrhizas they remained stunted. Growth accelerated as soon as these structures began to appear, however, presumably with the development of effective amounts of fungal inocula which may have been initially absent.

Until now the benefits accruing to hosts after mycorrhizal formation—increasing root and stem production[3]—have usually been attributed to enhanced mineral nutrition[4]. Marx and Bryan found that stems of trees with mycorrhizas grew taller and wider than those of trees without mycorrhizas[5]. In recent experiments, however, with (1) seedlings of one seedlot of birch (*Betula verrucosa* Ehrh.) and (2) two isolates of one of its ectomycorrhizal associates *Amanita muscaria* (L. ex Fr.) Hooker, it was found that different stem tissues were affected differently. Inoculating 10-d-old seedlings growing axenically on modified Ingestad's medium[6] solidified with 1% agar, with isolates 1 and 2 taken from sporophores collected at Tomintoul and Penicuik, Scotland (Table 1) significantly increased stem diameters by 22 and 31% respectively when assessed 8 weeks later.

Inoculation, which resulted in the formation of mycorrhizas within 4 weeks, increased widths of epidermal layers by 400 to 500%, but those of vascular tissues by only 6 to 18%. But when transverse sections were examined microscopically, in addition to affecting the widths of differing bands of tissue, inoculation was found to have altered their nature (Table 2).

Table 2 Differences in stem anatomy of seedlings of *Betula verrucosa* with and without mycorrhizas formed with *Amanita muscaria*

Seedlings without mycorrhizas	Seedlings with mycorrhizas
Epidermis one cell thick without bark	Bark well developed, five cells thick
Lenticels absent	Lenticels developing
Cortex—small, thin-walled cells, closely adpressed, with very few intercellular spaces	Cortex—large cells with conspicuously 'thickened' walls, rounded with many distinct intercellular spaces
Phloem parenchyma—very infrequent occurrence of starch	Phloem parenchyma—most cells with starch and darkly staining substances
Xylem—secondary thickening patchily distributed	Xylem—uniform secondary thickening throughout tissue
Primary tissues—virtual absence of starch in pith	Primary tissues—most pith cells contain starch grains

Just as when pot-grown tomato plants were infected with the vesicular-arbuscular fungus *Glomus macrocarpus* var. *geosporus*[7], so inoculation of *B. verrucosa* with *A. muscaria* was similarly associated (Fig. 1) with (1) the more uniform and probably thicker secondary thickening of the xylem, (2) larger and more rounded cortical cells with conspicuous intercellular spaces, (3) the relatively advanced development of bark, which was missing from non-mycorrhizal controls and (4) increased starch content of the phloem parenchyma and pith. Where it occurred, bark was punctuated with lenticels.

What advantages do these morphological changes, probably attributable to changed hormonal balances, confer on young trees, in view of the fact that they may reflect the ability of mycorrhizas to accelerate processes which might occur inevitably with increased age? They seem to be of two kinds related to (1) gaseous exchange, and (2) structural properties.

The effect of *A. muscaria* on stem cortex development can be regarded as an extension of a phenomenon identified by Coutts (see ref. 1, page 376). They found that air spaces

Table 1 Effects of inoculating roots of *Betula verrucosa* with the mycorrhizal *Amanita muscaria* on the width of stem tissues

	Width of stem tissues (μm)			
Treatment	Epidermis/bark	Cortex	Xylem/pith	Total
Uninoculated control	16	100	514	629
Inoculated with:				
isolate 1	100 (530)	121 (22)	543 (6)	765 (22)
isolate 2	85 (430)	138 (38)	604 (18)	827 (31)

Effects were assessed after 8 weeks of growth in axenic conditions at 26 °C with continuous light (mycorrhizas appeared 4 weeks after inoculation). Measurements were made on three sections of each replicate seedling. Figures in brackets refer to percentage increases.

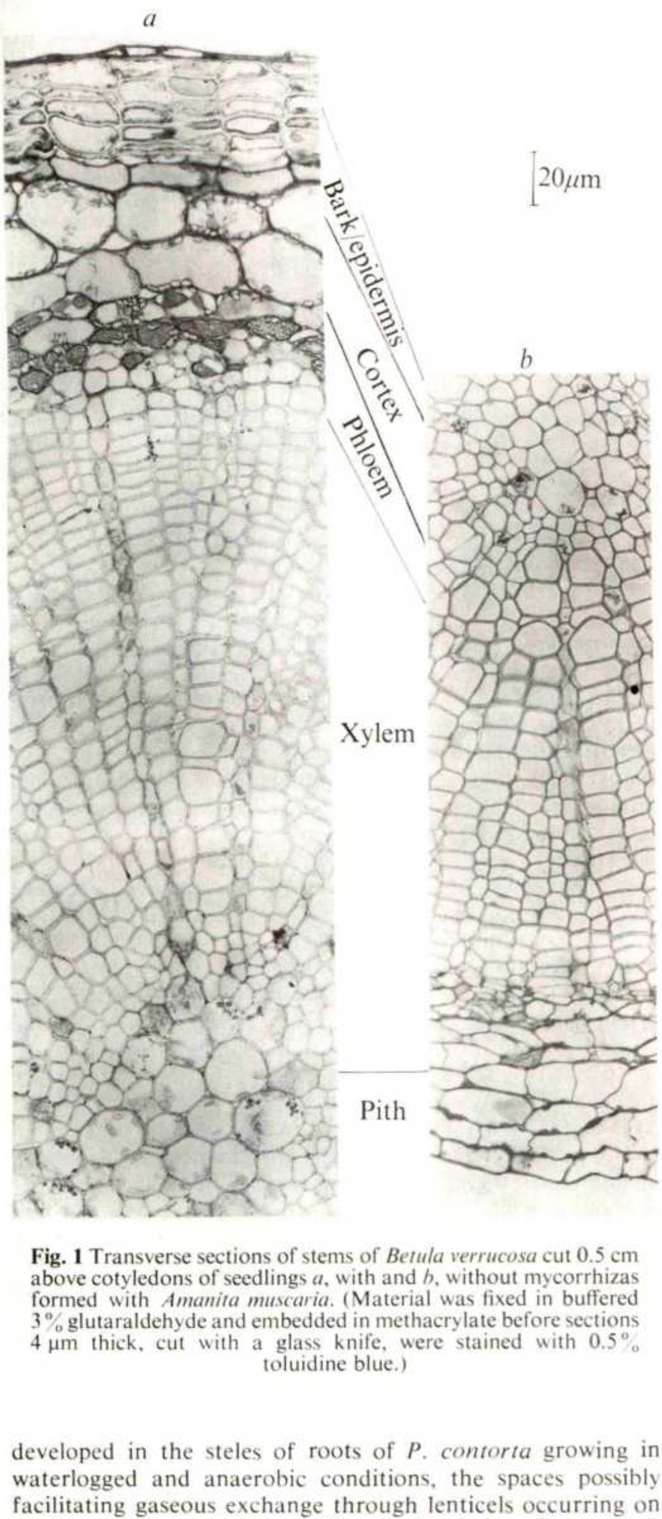

Fig. 1 Transverse sections of stems of *Betula verrucosa* cut 0.5 cm above cotyledons of seedlings *a*, with and *b*, without mycorrhizas formed with *Amanita muscaria*. (Material was fixed in buffered 3% glutaraldehyde and embedded in methacrylate before sections 4 μm thick, cut with a glass knife, were stained with 0.5% toluidine blue.)

developed in the steles of roots of *P. contorta* growing in waterlogged and anaerobic conditions, the spaces possibly facilitating gaseous exchange through lenticels occurring on sections of root and stem. The accelerated appearance of bark and lenticels and the concomitant development of a spongier cortex in stems of *B. verrucosa* may serve a similar purpose, stem changes possibly complementing cortical alterations occurring in roots with sheathing mycorrhizas.

On the other hand, accelerated bark development might hasten the waterproofing of trees which otherwise might be at risk in arid conditions. Zerling, as mentioned in a review by Meyer[8], found that sheathing mycorrhizas improved the water relations of trees in prairie and steppe regions.

Thus, while cortical changes in stems might facilitate tree establishment in 'stress conditions', whether these are associated with too much or too little water, the greater thickening of xylem elements and the early development of bark may confer structural benefits, not only increasing quality (uniformity) and quantity of yield but also possibly aiding the ability of the tree to withstand exposure.

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Phytochrome-initiated fast rhythm controlling developmental response

To identify the steps controlling a developmental response, we must relate changes occurring soon after the inducing signal to responses many hours later. In higher plants exposure to red light shifts developmental patterns by converting phytochrome, a ubiquitous plant pigment, into its active form[1]. Far red light converts phytochrome back to its original form and stops the response. Red light induces changes in electrical properties of plants in 15–30 s (refs 2, 3) and within minutes ATP levels[4], NADP transport[5], and nitrate reductase activity[6] change. It requires hours or even days for phytochrome effects on circadian rhythms[7], germination, de-aetiolation, and flowering responses to appear[1]. Until now there has been no indication of the mechanism by which events immediately following phytochrome conversion determine the later development. It should be possible to probe the nature of the first controlling steps by perturbing these steps and thus affecting growth. If the steps directly following phytochrome conversion are sensitive to additional red light, then a second illumination will alter the overall growth. We have found that a brief flash of red light given to dark-grown bean seedlings starts oscillations in a temperature-compensated system. The interaction of this system with a second flash of red light showed that the oscillating system controls subsequent development.

Compared with unilluminated plants, bean seedlings (*Phaseolus vulgaris* var. Black Valentine) exposed to 10 min of white light and returned to dark have 40% greater leaf protein and leaf weight 24 h later[8]. Red illumination, 115 $\mu W\ cm^{-2}$ at 600 nm, saturated this growth response in 4 min, while our experiments used a total of 8 s of this red light. Given as a single flash, 8 s of red light increases average leaf weight by 8%. We used leaf weight increase in whole seedlings to measure the integrated phytochrome response.

To determine the effects of interference by a second red light on the reaction to the first, we kept total illumination constant and varied the interval between two 4-s flashes. For intervals ranging from 2 min 0 s to 2 min 30 s in increments of 5 s, we found that the length of the interval determined the leaf weight 24 h later. In 11 trials minimum growth occurred in the samples exposed to an interval around 2 min 15 s and maximum growth for an interval only 5 s longer. The maximum weight increase was 60% greater than the minimum. This sharp jump from low to high weight increase is an easily observable feature to use in studying the interacting response to red light.

Given the repeatable difference in growth response to intervals of 2 min 15 s and 2 min 20 s, does the same minimum–maximum jump occur for any other pair of intervals 5 s apart? We extended the range of intervals from 0 min 5 s to 5 min 15 s and recorded average weight as a function of interval (Fig. 1). Arrows mark every significant minimum–maximum jump where probability from analysis of variance is less than 0.05. After the jump at 2 min 15 s to 2 min 20 s, the next significant one is at 3 min 0s to 3 min 5 s, 45 s later. The likelihood of finding the next three jumps spaced at 45±5 s intervals from these is only $3^3(3!\,24!)/27! = 0.009$. The part of the curve between the significant transitions shows hints of repetition but no outstanding features. Autocorrelation shows pronounced periodicity for 45 s, $P<0.05$, and 90 s, $P<0.01$. The 45-s periodicity persists at least 20 min. For intervals as long as 20–22 min, the minimum–maximum jump in the response appears at 20 min 20 s to 20 min 25 s and 45 s later at 21 min 5 s to 21 min 10 s.

Apparently red light shifts some system closely associated with controlling the growth rate into a rapidly oscillating state. If the changing sensitivity to a second illumination started by the initial red light is part of the phytochrome response, then far red light given immediately after the first red should eliminate the minimum–maximum jumps in response to the second red. There is no minimum–maximum jump at any interval when 4 s far red immediately follows the first red (Fig. 2). Far red light must either be damping the oscillations or desynchronising the plants so that the minimum–maximum jumps are no longer distinct. Variations among individual plants for any interval is no greater for initial red–far red than for red light alone, indicating that far red damps or stops the oscillations.

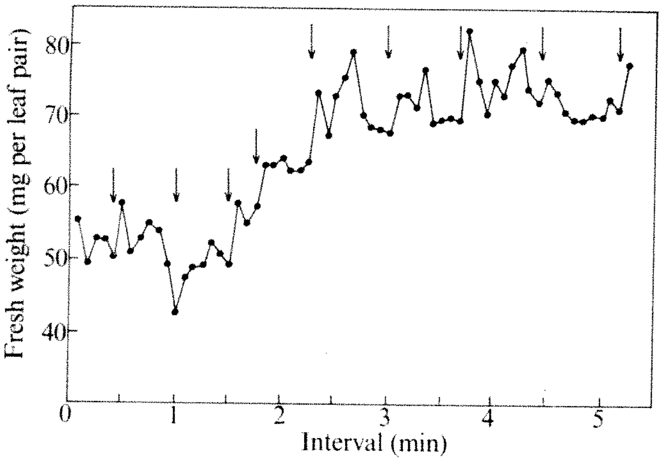

Fig. 1 The effect on leaf growth of the interval between two 4 s red illuminations. Average weight of primary leaf pairs from 8-d-old seedlings 24 h after red light. Arrows mark all the intervals where the second red light significantly suppresses growth compared to the interval 5 s longer, the minimum–maximum jumps. Plants were grown in the dark with standard nutrient: 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 1 mM $MgSO_4$. Six pots of 15 seedlings each were given two 4 s red light pulses, 115 µW cm^{-2}, with a measured dark interval between. The standard deviation among the samples for each interval ranges from 3 to 13%. For comparison, % std average fresh leaf weight: 7 d unilluminated, 9%; 8 d unilluminated, 5%; and 8 d with 10 min red light on day 7, 6%.

To prove that the rapidly oscillating system is phytochrome dependent, we must show repeated reversibility, with red light reversing the damping effect of far red. When red immediately follows far red it re-establishes the minimum–maximum jumps in response to the final red signal (Fig. 3). Arrows mark the significant jumps at 2 min 5 s to 2 min 10 s and 3 min 15 s to 3 min 20 s in two replicate experiments. The shape of the curve is unlike the shape with red alone, but minimum–maximum jumps in the response are clearly not damped out as they are by far red light.

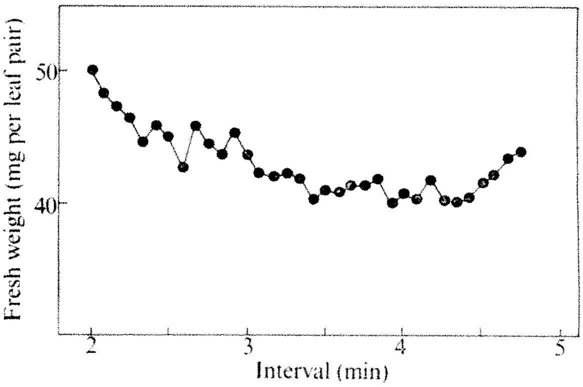

Fig. 2 Far red reversal. The effect on leaf growth of the interval between 4 s red light followed immediately by 4 s far red light, 2500 µW cm^{-2}, and another 4 s red illumination after the dark interval. Other conditions as in Fig. 1.

The period of the oscillation is independent of temperature between 15 and 30 °C. The seedlings were grown, and experiments described previously were carried out at 22 °C. To avoid the possibility that a temperature shift itself might produce time-dependent effects, we moved seedlings into the 15 or 30 °C room at random times with respect to illumination. At 15 °C, the entire plants equilibrated to 15 °C. Minimum–maximum jumps in the response occurred 40 or 45 s apart between 3 min 0 s and 6 min 0 s. At 30 °C, hypocotyls equilibrated to 30 °C, but transpiration and evaporation lowered the leaf temperature to 25 °C. For intervals ranging from 2 min 0 s to 4 min 0 s, there were three minimum–maximum jumps in the response, 45 and 40 s apart. The period of the oscillation is unchanged, but the phase of the oscillation is affected by the temperature during the illumination.

The results demonstrate that we can study the system controlling the phytochrome response by perturbing it with additional red light. The presence of oscillations shows that previous models[9], which give the developmental response as a monotonically increasing function of the inducing red light, must be revised for subsaturating amounts of light. Initiation of these oscillations is among the first consequences of phytochrome conversion. The system is active

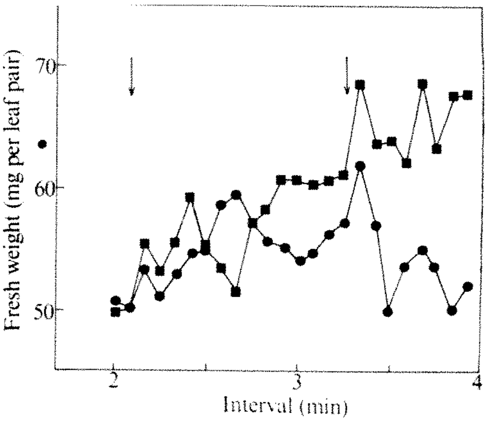

Fig. 3 Red reversal of far red. The effect on leaf growth of the interval between 4 s red light, 24 s far red, and 4 s red, followed by the dark interval and then another red illumination. Arrows mark the minimum–maximum jumps. Other conditions as in Fig. 1.

within 10 s, since growth responses to the second flash of red light at 5 and 10 s are significantly different, $P<0.01$. It is certainly functioning within 25 s, even though it does not reach stable oscillations for 2 min. The temperature independence of the system implies that it is in some organised structure. The system must be linked to, but is probably not identical to, the phytochrome binding sites in the membranes. Since circadian rhythms in plants are sensitive to phytochrome and are temperature compensated, this oscillating system may be the mechanism by which red light interacts with those rhythms. The growth response to two red light signals shows that phytochrome conversion rapidly activates a new type of control linking early events to later development through an oscillating system.

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Two previously undescribed potato viruses from South America

THE proneness of potatoes (*Solanum* spp.) to virus diseases is well known, and about 20 viruses are recorded in naturally infected potatoes in Europe and North America[1,2]. Many of these viruses also occur in the Andean region of South America. For example, in Peru several well known viruses were detected[3] in 'basic seed' stocks by inoculating sap to a range of indicator species and by serological tests. Some indicator plants in these tests, however, developed symptoms unlike those typical of previously described potato viruses. These symptoms were of two main types, necrotic local lesions and systemic necrosis in *Chenopodium quinoa*, and systemic necrosis and shoot distortion in *Phaseolus vulgaris* cv. Pinto or Sanilac. We describe here the characterisation of the virus isolates producing these effects. Two previously undescribed viruses were identified: we call them potato black ringspot virus (PBRV) and potato virus T (PVT).

The stock culture of the first virus, PBRV, is an isolate obtained from a plant of the cultivar Antarqui (*S. tuberosum* subsp. *tuberosum* × *S. tuberosum* subsp. *andigena*) growing at Huasa Huasi, Junin, Peru and showing necrotic spotting on the tip leaves. In glasshouse conditions at Dundee, PBRV produces local lesions in inoculated *C. quinoa* leaves in 4 d and systemic necrosis in 6–8 d. Necrotic local lesions develop in 6 d in *P. vulgaris* cv. The Prince; systemic necrotic spotting is produced in 7–8 d and ultimately the growing point of the main shoot is killed. Potato plants (*S. tuberosum* subsp. *tuberosum* cv. Maris Bard) infected by inoculation of sap develop black rings and spots in systemically infected leaves. PBRV has a very wide host range, which will be described in detail elsewhere, occurs in moderate concentration in sap and is moderately stable (Table 1). Purified virus preparations, made from systemically infected *Nicotiana clevelandii* leaves by the method of Murant *et al.*[4], contain numerous isometric particles which measure about 25 nm in diameter in electron micrographs of samples negatively stained with phosphotungstate. Gel-diffusion serological tests indicate that PBRV is distantly related to tobacco ringspot virus (Table 2) and therefore belongs to the nepovirus group[5], other members of which have soil-inhabiting nematodes as vectors and are transmitted through the true seed of many plant species.

Table 1 Some properties of PBRV and PVT

Properties in sap*	PBRV	PVT
Dilution endpoint	10^{-4} to 10^{-5}	10^{-4} to 10^{-5}
Thermal inactivation point (10 min exposure)	58–60 °C	55–60 °C
Longevity at 20 °C	9–10 d	2–4 d
Particle shape and size (nm)	Isometric (25)	Flexuous filament (640 × 12)

*Source and assay plants respectively were *N. clevelandii* and *C. amaranticolor* for PBRV, and *C. quinoa* and *P. vulgaris* cv. Pinto for PVT.

Table 2 Serological relationship between PBRV and tobacco ringspot virus

Antiserum	Antigen* PBRV	Antigen* Tobacco ringspot virus
PBRV	1/2048	1/32
Tobacco ringspot virus	1/16	1/512

*Figures are antiserum reaction end-points in gel-diffusion serological tests.

The stock culture of the second virus was also obtained from potato cv. Antarqui growing at Huasa Huasi, but the source plant showed no obvious symptoms of infection. This culture was passed through a series of local lesions, which develop in heavily shaded inoculated leaves of *P. vulgaris* cv. The Prince in 6 d; systemic vein necrosis is produced in 10 d, and leaves formed subsequently show a distorting mosaic. In *C. quinoa* PVT is much less virulent than PBRV, producing no symptoms in inoculated leaves and a mild systemic mottle after 10 d. Typically, no symptoms develop in potato cv. Maris Bard infected by inoculation of sap. PVT is less stable than PBRV in sap at 20 °C (Table 1) and has a narrower host range. Infective *C. quinoa* sap, negatively stained with uranyl acetate, contains flexuous filamentous particles measuring about 640 × 12 nm. Serological tests made by the tube precipitin method, using virus particles purified by a technique similar to that of Lister[6], and antisera to purified virus, indicate that PVT is related to apple stem grooving virus (Table 3) but not to potato viruses S or M. Significantly PVT is frequently seed-transmitted in various solanaceous species; for example, of 18 progeny seedlings derived from experimentally infected *Datura stramonium* mother plants, 13 were infected.

Our report is intended to alert potato pathologists to the occurrence of PBRV and PVT, and to indicate how

Table 3 Serological relationship between PVT and apple stem grooving virus

Antiserum	Antigen* PVT	Antigen* Apple stem grooving virus
PVT	1/256	1/64
Apple stem grooving virus	1/32	1/128

*Figures are antiserum reaction end-points in tube precipitin tests, after incubation for 5 h at 37 °C.

they can be detected. Both viruses seem to be widespread in Peru. Our findings also support the idea that the Andean region of South America can be considered the main centre of diversity not only of tuber-bearing species of *Solanum*[7] but also of their viruses. Early export of infected potato tubers from South and Central America to various other countries is probably responsible for the present worldwide distribution of many potato viruses. Other viruses, which did not occur in the originally exported material or did not spread in the new country, are likely still to be confined to the Andean region. Indeed we have evidence of viruses in addition to PBRV and PVT that occur in South American potatoes but have not been reported elsewhere.

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Control of inner cell mass development in cultured mouse blastocysts

THE mouse blastocyst consists of a group of cells, the inner cell mass (ICM), attached to a small area of the inside surface of a sphere of trophectoderm cells. The trophectoderm gives rise to the extraembryonic regions of the conceptus while the ICM forms the embryo proper[1]. At implantation trophoblast cells invade the uterine epithelium. Those overlying the ICM proliferate to form the ectoplacental cone and the extraembryonic ectoderm and the remainder undergo transformation into giant cells[2]. The cells of the inner cell mass divide further, and, together with the extraembryonic ectoderm, give rise to a column of cells, the egg cylinder, that extends into the blastocyst cavity (blastocoele). Lactic dehydrogenase A subunits (4A, LDH-5) appear at this time[3], and may be synthesised first in tissue deriving from the ICM[4]. Subsequently LDH-4 (3A1B) and LDH-3 (2A2B) appear as the contribution of B subunits to the pattern increases. If implantation is prevented, further development of the blastocyst is arrested[5] and LDH-5 fails to appear[3]. Implantation therefore seems to act as a trigger, not only for trophoblast proliferation, but also, directly or indirectly, for further development of ICM. In the work reported here we have varied the conditions of culture of isolated blastocysts and have elicited alternative forms of development that mimic those observed *in vivo* when implantation occurs or is prevented. We have shown further that the triggering of ICM development *in vitro* may represent the release from an inhibitory effect of trophectoderm.

Blastocysts flushed from the uteri of Q strain mice on the fourth day of pregnancy were cultured in supplemented Eagle's minimal essential medium with 20% foetal calf serum[4] in various conditions intended to favour or inhibit attachment of the blastocysts to the substratum. The results obtained are summarised in Table 1 and illustrated in Fig. 1. On Falcon dishes, attachment and outgrowth begin asynchronously on the second day of culture and all blastocysts are outgrown by the third day, showing giant cell mass grew vigorously. Such clumps of blastocysts were neighbouring trophoblast cells. As shown in Table 1 (group *A*) such outgrowths synthesise substantial amounts of LDH-5. Groups *B–D* give the results obtained with three different suspension culture conditions intended to inhibit blastocyst attachment. These were the use of substrata unfavourable to blastocyst attachment and outgrowth (bacteriological or agar-coated dishes), and hanging drop culture of single embryos, either on plastic cover slips suspended in oil, or in inverted Falcon microtest plates with the wells filled to capacity (15 μl) with medium. In each case synthesis of LDH-5 is greatly reduced. None of these conditions was completely reliable in preventing attachment during 3 d of culture. Blastocysts did eventually attach to the bacteriological dish or agar surface, and, in hanging cultures, to the oil–medium interface or to the air–medium interface. In all cases such attachment led to the disappearance of the blastocoele cavity and, rarely, to some increase in the mass of cells. Such 'collapsed' blastocysts often contained some LDH-5, presumably depending on the time of blastocyst 'attachment' and/or collapse relative to the time of collection. Attachment to the agar surface was undoubtedly responsible for the three batches of embryos in group *B* (Table 1) that synthesised significant amounts of LDH-5. Even more strikingly, if groups of blastocysts were cultured together in a hanging drop, they always coalesced rapidly, their blastocoele cavities disappeared and the total cell transformation and proliferation of the ICM and invariably found to be positive for LDH-5 (gel 4, Fig. 1). When blastocysts that had remained in suspension for 3 d were transferred to Falcon dishes, attachment and outgrowth began within 15 min in a very synchronous fashion and significant amounts of LDH-5 were synthesised by the following day (gel 6, Fig. 1).

Since these results suggested that further development is related in some way to a collapse of the trophectoderm, we

Table 1 LDH-5 synthesis in blastocysts and ICMs after 3 or 4 d of culture in different conditions

Group	Culture conditions	No. of batches of embroyos*	No. of batches with LDH-5 band				
			—	±	+	++	+++
A	Blastocysts (outgrowths) on Falcon dishes	16	0	0	0	5	11
B	Blastocysts (suspended) on agar-coated dishes	9	6	2†	0	1†	0
C	on bacteriological dishes	2	1	0	1	0	0
D	singly, in hanging drops	11	7	4	0	0	0
E	ICMs (suspended) on bacteriological dishes	2	0	0	0	0	2
F	singly, in hanging drops	2	0	0	1‡	0	1

* An average of eight embryos per batch was applied on each microgel.
† Attached.
‡ Two ICMs only.

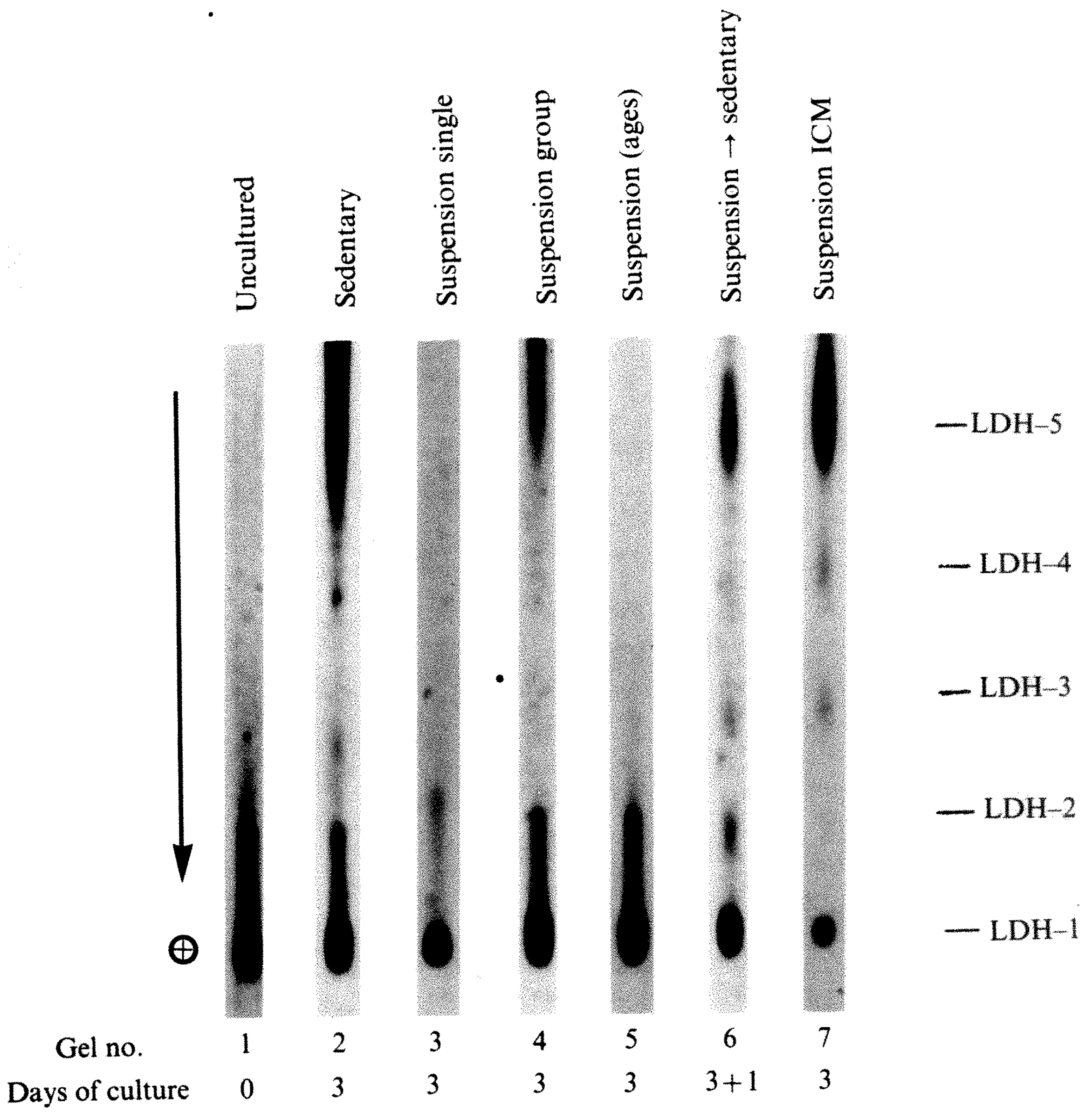

Fig. 1 Microdisk electrophoresis showing LDH isozymes in extracts of embryos after sedentary and suspension culture from the blastocyst stage. Isolation of blastocysts on the fourth day of pregnancy, media, and LDH staining were as described before[4]. Zonae pellucidae were removed before culture by 5 min of incubation in pronase. Polyacrylamide gel electrophoresis in 5-μl Drummond caps was performed as described before[7]. Approximately 10 embryos were applied to each gel as follows: (1) uncultured blastocysts, (2) outgrowths from blastocysts after 3 d of sedentary culture on a Falcon dish, (3) blastocysts after 3 d of suspension culture singly in hanging drop, (4) blastocysts after 3 d of suspension as a group in hanging drop, (5) blastocysts after 3 d of suspension culture on an agar-coated dish, (6) outgrowths from blastocysts cultured for 3 d on an agar-coated dish followed by 1 d of culture on a Falcon dish, (7) inner cell masses after 3 d of culture in suspension on a bacteriological dish. All extracts contain residual maternally-inherited LDH-1 (ref. 4).

analysed isolated ICMs in suspension culture. Trophectoderm was removed immunosurgically[6] by treatment of zona-free blastocysts with rabbit anti-mouse serum followed by guinea pig complement. Trophectoderm cells are lysed; ICMs, because they are not directly exposed to the anti-mouse serum, survive. Such ICMs grow and develop in suspension culture, forming a layer of primary endoderm cells on their surface after 2 d. In contrast to whole blastocysts, they synthesise LDH-5 in the absence of attachment and outgrowth (Fig. 1 and Table 1, groups *E* and *F*). This result suggests that implantation relieves an inhibitory effect of trophectoderm on ICM development. This inhibition could be due to a restriction on free access for some nutrient(s), or to a more subtle contact effect.

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β-retinoic acid inhibits and reverses testosterone-induced hyperplasia in mouse prostate organ cultures

CERTAIN human prostatic cancers are associated with abnormalities of testosterone metabolism and/or increased sensitivity of prostatic epithelial cells to testosterone[1,2]. Most treatments, (oestrogen therapy, orchiectomy and radiation therapy) for these diseases are designed therefore to reduce the levels of testosterone or its active metabolites in the patients[3]. Recently, however, studies with

Table 1 Inhibition of testosterone (1.8×10^{-5} M)-induced hyperplasia (m.i. ±s.d.)*† by different concentrations of β-retinoic acid (RA)

Days after treatment	Control	Testosterone (1.8×10^{-5} M)	Cultures were simultaneously treated with testosterone (1.8×10^{-5} M) and different concentrations of RA from the beginning of the experiment		
			RA (1.7×10^{-5} M)	RA (3.3×10^{-6} M)	RA (6.6×10^{-7} M)
4	148±59	647±261 $P<0.01$‡	147±45 $P<0.01$§	148±25 $P<0.01$§	389±87
8	145±68	637±185 $P<0.01$‡	218±81 $P<0.01$§	340±136	239±28 $P<0.05$§

*The number of arrested metaphases per 10^5 cells during a 4-h period of colcemid treatment.
†Mean and s.d. of 6–8 explants per group.
‡Mitotic stimulation significant as compared to the corresponding control group.
§Mitotic inhibition significant as compared to the corresponding group that received only testosterone.

non-hormonal chemotherapeutic agents for the treatment of prostate cancer have been initiated[3-5]. In mouse prostate organ cultures, we and Lasnitzki have shown that vitamin A and several synthetic analogues (retinoids) inhibit and reverse carcinogen-induced epithelial lesions[6-8]. Since testosterone has been linked with human prostatic cancer and induces hyperplastic lesions of the prostatic epithelium *in vitro*[2,9], it prompted us to start investigating the effects of β-retinoic acid (RA) and other retinoids on testosterone-induced lesions in the mouse prostate system. Preliminary results presented in this report show that RA inhibited and reversed testosterone-induced lesions in mouse prostate organ cultures.

Ventral prostate from 8- to 10-week-old C3H mice were removed aseptically and teased into approximately 1.5×1.0-mm explants. The explants were arranged on strips of lens paper and were placed on stainless steel grids. Six to eight explants were placed on each grid that were contained in 35 mm plastic Petri dishes. Petri dishes were filled up to the level of the grids with CMRL 1066 medium (~2.5 ml) supplemented with 10% horse serum, 3% chick embryo extract, 100 U ml^{-1} each of penicillin and streptomycin and 25 μg ml^{-1} amphotericin B. The cultures were incubated at 37 °C in an atmosphere of 50% O_2+45 N_2+5CO_2. Culture medium was renewed every 48 h.

Two series of experiments were carried out. In the first, explants were incubated simultaneously with testosterone (1.8×10^{-5} M) and different concentrations of RA to determine if the development of hyperplasia could be prevented. In the second, the reversal of hyperplasia was examined. In these experiments, explants were first allowed to develop hyperplasia by treatment with the testosterone for 8 days, after which time, these hyperplastic explants were simultaneously treated with testosterone and different concentrations of RA for an additional 96 h. The changes in rates of cell proliferation caused by testosterone or RA were determined by the colcemid metaphase arrest technique[6]. The explants were fixed in buffered formalin, processed for histology, serially sectioned, and the mitotic index and standard deviation (m.i.±s.d.) determined as previously described[6]. The significance of the difference between two groups was determined by Student's *t*-test, and a value of $P<0.05$ was considered significant.

Since the mitotic indices in different groups of explants were variable, the data presented in each table represent one experiment. Table 1 shows that testosterone stimulated cellular proliferation in the epithelium of prostate at 4 or 8 days after treatment, but that this stimulatory effect was prevented in explants that were simultaneously treated with testosterone and RA. For instance, at 4 d after treatment, the m.i. in the control explants was 148±59, whereas the corresponding value in testosterone-treated explants was 647±261. The m.i. in explants simultaneously treated with testosterone and RA were 147±45, 148±25 and 389±87 at 1.7×10^{-5} M, 3.3×10^{-6} M, and 6.6×10^{-7} M, respectively. A similar mitotic inhibitory effect by RA was observed at 8 d.

The morphology of prostate explants incubated in control media for 8 d showed little alteration from the *in vivo* structure. The alveolar epithelium remained 1–2 cell layers thick and exhibited little mitotic activity. Occasionally some Periodic-Acid Schiff (PAS) positive secretory material was present in the alveolar lumen of the control explants. Treatment of explants with testosterone produced hypertrophy and hyperplasia of the alveolar epithelium. The epithelium of these explants was tall, contained many mitoses, and showed a significant increase in the PAS positive material in the alveolar lumen. When, however, explants were treated simultaneously with the testosterone and RA (1.7×10^{-5} M), the degree of hypertrophy and hyperplasia was significantly less than that of explants that received the hormone only.

Table 2 shows the data on the reversal of testosterone-induced hyperplasia by RA and indicates that testosterone stimulated proliferation in the prostatic epithelium at 8 d, and that the values of m.i. in the testosterone-treated

Table 2 Reversal of testosterone (1.8×10^{-5} M)-induced hyperplasia (m.i. ±s.d.)*† by different concentrations of β-retinoic acid (RA)

Days after treatment	Control	Testosterone (1.8×10^{-5} M)	Cultures were treated with testosterone (1.8×10^{-5} M) for 8 d, followed by simultaneous treatment with testosterone and different concentrations of RA			
			Hours after adding β-retinoic acid	Testosterone + RA (1.7×10^{-5} M)	Testosterone + RA (3.3×10^{-6} M)	Testosterone + RA (6.6×10^{-7} M)
8	64±14	556±146 $P<0.01$‡				
10	107±56	753±127 $P<0.01$‡	48	133±59 $P<0.01$§	103±45 $P<0.01$§	185±48 $P<0.01$§
12	140±53	602±227 $P<0.01$‡	96	95±18 $P<0.01$§	125±30 $P<0.01$§	164±60 $P<0.01$§

*The number of arrested metaphases per 10^5 cells during a 4-h period of colcemid treatment.
†Mean and s.d. of 6–8 explants per group.
‡Mitotic stimulation significant as compared to the corresponding control group.
§Mitotic inhibition significant as compared to the corresponding group that received only testosterone.

explants remained significantly higher than the corresponding control values for at least 12 d. But when explants were first treated with testosterone for 8 d followed by simultaneous treatment with testosterone and RA at 1.7×10^{-5} M, 3.3×10^{-6} M, and 6.6×10^{-7} M for an additional 48 or 96 h, the hyperplasia was reversed.

The mechanism of inhibition and reversal by RA of testosterone-induced hyperplasia in the mouse prostate is not known. It has been shown that steroid hormones interact with their specific proteins in the target tissues. In testosterone-dependent tissues for instance, the prostrate gland of experimental animals[10–12] and humans[13], the existence of proteins with a pronounced affinity for the hormone has been documented. Furthermore, for its biological activity, testosterone must be metabolised (mediated by the enzyme 5α-reductase) to 5α-dihydrotestosterone, the form that appears to be responsible for the stimulation of proliferation of the prostatic epithelium[3,14–16]. Thus the inhibition of binding of testosterone and/or its metabolism to 5α-dihydrotestosterone would result in its failure to cause stimulation of proliferation. Sandberg *et al.*[3] suggested that oestrogens, which have a remarkable efficacy in alleviating human prostatic carcinomas, act by reducing the testosterone titres directly via the pituitary[17–19] and/or indirectly by inhibiting the enzyme 5α-reductase[20,21]. Since the prostate organ culture model used in the present investigation does not involve the pituitary, the inhibition of testosterone-induced hyperplasia by RA may be accomplished by inhibition of the binding of testosterone to the specific protein and/or by inhibition of the metabolism of testosterone. However, the antihyperplastic activity of RA can also be explained by several other mechanisms.

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Virulence and toxicity of axenic *Entamoeba histolytica*

The ability of cultured *Entamoeba histolytica* to cause ulcers in the caecum of guinea pigs and rats or abscesses in the liver of the Syrian hamster decreases rapidly on elimination of the accompanying bacterial flora[1–4]. It seemed that some strains grown axenically on TP-S-1 medium[5] for many years had lost their pathogenicity entirely. When very large inocula were used, however, (10^7 amoebae), a granulomatous lesion was evoked in the hamster liver[6,7]. Our experiments with strains made freshly axenic, isolated from patients with amoebic dysentery, suggested that the number of amoebae required to induce a certain extent of pathology in the hamster liver increased with the number of transfers in axenic culture[8]. After a long period of axenic growth, strains probably maintain a very low degree of pathogenicity. Using two such strains, HK-9 and HB-301, we obtained results which may contribute to the understanding of the pathogenicity of *E. histolytica*. We were able to restore the virulence of the HK-9 strain with cholesterol and establish a higher degree of virulence by two successive passages through hamster liver (HK-9H_2). *In vitro* toxicity towards guinea pig leukocytes, and agglutinability induced by concanavalin A (con A) were markedly greater in strain HK-9H_2 than in HK-9. Antigenic analysis revealed a pattern for HK-9H_2 different from that of the HK-9 strain.

Table 1 Virulence* of *E. histolytica* HK-9H_2 to hamster liver

Time since last passage (weeks)	Size of inoculum ($\times 10^5$ amoebae in 50 μl)			
	0.5	2.5	4.5	8
4	7	49	—	—
11	—	—	—	100
17	0	8	—	—
34	—	—	8	18

*Virulence is expressed as the average extent of abscess as a percentage of the infected central liver lobe. Between five and 10 hamsters were used in each experiment. They were killed 1 week after infection.

Amoebae were injected, after laparotomy of the hamster under anaesthesia with ether, through a 20-gauge needle. The volume of the inoculum chosen was as small as possible to avoid damage of the liver parenchyma. Virulence studies are detailed in Tables 1 and 2. On intrahepatic injection of 10^7 amoebae in 150-μl-inocula, strain HK-9 showed no virulence, whereas HB-301 caused small abscesses occasionally. Two transfers on TP-S-1 medium to which cholesterol (0.35 mg per tube) was added increased the virulence of the HK-9 strain. Inocula of 5×10^6 amoebae induced small granulomatous lesions from which amoebae could sometimes be cultured axenically. The virulence of the resulting HK-9H_1 strain, that is HK-9 after one passage through hamster liver, was markedly increased. Further passages through hamster liver increased virulence even more, so that inocula as low as 5×10^4 sometimes caused abscesses. After 16 weeks virulence was reduced, however, with larger inocula required to induce lesions (Table 1).

Further experiments confirmed the effect of cholesterol on the virulence of the HK-9 strain (Table 2). Cholesterol did not further increase the virulence of HB-301 strain. Epicholesterol—the 3α-hydroxy isomer of cholesterol which, unlike cholesterol, does not induce changes in the permeability of the cell[9]—had the same effect. Although amoebae were present in the small abscesses they could be cultured on TP-S-1 medium only twice; the number of amoebae was probably too small to initiate an axenic culture in other cases. When the hamsters were not killed after 1 week, but allowed to live on their normal diet of food pellets and water, the abscesses healed and eventually disappeared. An effect of cholesterol on the virulence of axenic amoebae has not been reported before. There is disagreement on the effect of cholesterol on the invasiveness of amoebae-bacteria cultures in the rat caecum[10–11].

Virulent HK-9H_2 and non-virulent HK-9 strains were characterised by at least three clear differences—toxicity for guinea pig leukocytes, con A-induced agglutination,

Table 2 Effect of cholesterol and epicholesterol on the virulence of *E. histolytica* HK-9 and HB-301

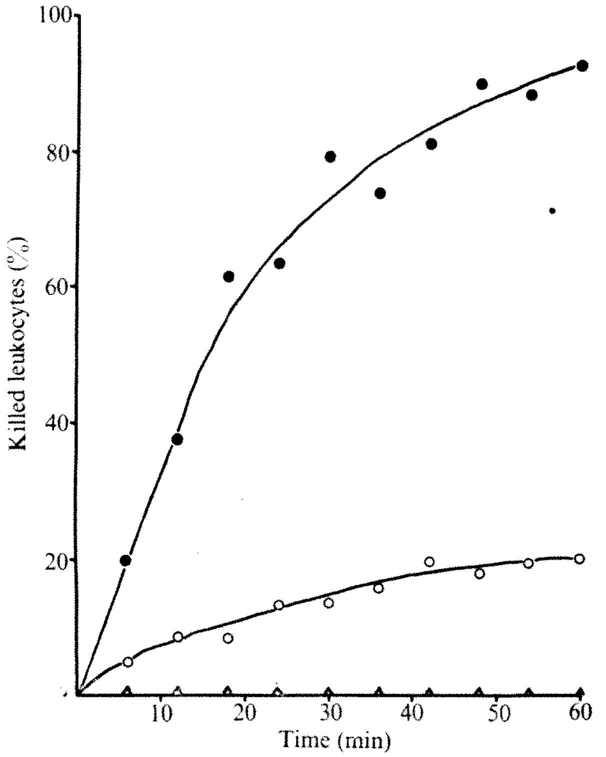
Strain	Treatment	No. of experiments	Size of inoculum ($\times 10^6$)*	No. of hamsters with abscesses per no. of hamsters inoculated	
HK-9	Untreated	3	1.1–4.8	0/12	(0%)
	Cholesterol	5	1.2–5.8	6/15	(40%)
	Epicholesterol	2	1.5–2.0	4/8	(50%)
HB-301	Untreated	2	1.7–3.6	4/6	(67%)
	Cholesterol	2	1.5	5/8	(63%)

Cholesterol and epicholesterol were dissolved in chloroform and evaporated in culture tubes (0.35 mg per tube) before adding the TP-S-1 medium.

*Amoebae in 75–150 µl. The largest inoculum tested consisted of 75 µl packed cell volume and 75 µl TP-S-1 medium. One week after infection lesions were usually more or less confluent granulomatous necrotic areas. Amoebae were demonstrated by microscopy or culture of smears, or in histological cross-sections.

and antigenic composition. Amoebae from 2- or 3-d-old cultures were washed in physiological saline twice and a suspension of 3×10^6 amoebae per ml was prepared. Leukocytes were obtained by draining the peritoneal cavity of a guinea pig of about 300 g 16 h after the injection of 55 ml of physiological saline. The suspension contained approximately 10^7 leukocytes per ml, of which half were polymorphonuclear and half mononuclear leukocytes (monocytes, macrophages and lymphocytes). On mixing leukocyte and HK-9H$_2$ amoebae suspensions, 50% of the leukocytes were killed in about 15 min (Fig. 1), so that each amoebae killed an average of about 1.7 leukocytes. The increasingly smaller chance of an amoebae hitting an intact leukocyte may explain the shape of the curve. Induction of contact-lysis by *E. histolytica* in human leukocytes and cell cultures has been reported before[4,12,13]. Amoebae of the HB-301 and HB-301H$_2$ strains showed the same degree of toxicity towards guinea pig leukocytes as HK-9H$_2$, whereas the original HK-9 strain seemed to have a very low toxicity. HK-9H$_2$ maintained its leukocytotoxicity on the same level even when its virulence decreased in the course of 34 weeks. Toxicity could not be increased in HK-9 amoebae by addition of cholesterol to the medium; at least one passage through hamster liver was required for restoration of toxicity. Interactions between amoebae and pure suspensions of guinea pig macrophages were also studied. Macrophages were separated from other cells in a peritoneal washout by using their adherence to glass. Macrophages seemed to be lysed by the amoebae just as quickly as the inflammatory exudate cells.

Fig. 1 Contact-lysis of guinea pig leukocytes by *E. histolytica* strain HK-9 (○) and HK-9H$_2$ (●). The control (△) was a suspension of leukocytes without amoebae. The ratio of leukocytes to amoebae was approximately 10:3. Samples of 15 µl of each suspension were mixed on a clean microscope slide, covered with a coverslip and sealed with vaseline. The slide was maintained at 37 °C on a heated microscope stage and observed with a ×40 phase contrast objective. The percentage of leukocytes killed (that is, showing rounding up, fusion of nuclear lobes and diminution of granules) was assessed every 6 min by counting 100 leukocytes at random. A vital stain was not used.



Martinez-Palomo *et al.*[14] reported selective agglutination of pathogenic strains of *E. histolytica* induced by con A, We did not observe a clear correlation between con A-induced agglutination and virulence towards the hamster liver with our axenic strains. Only when HK-9 was compared with HK-9H$_2$ was a marked difference found. The technique of Martinez-Palomo *et al.* was modified, using microtitre plates and stepwise increase of con A concentration. Amoebae suspension (200 µl) was put in the cups of the microtitre plate and 10-µl drops of con A solution of 200 µg ml^{-1} were added one by one. After addition of a drop the suspension was shaken on a microshaker for 15 s and agglutination was assessed (0, +, ++, and +++) at a low magnification on the stage of an inverted microscope. Immediately after the second passage through hamster liver maximal agglutination was obtained in a suspension of HK-9H$_2$ amoebae of 10^6 ml^{-1} with con A at 15 µg ml^{-1}, whereas 40 µg ml^{-1} was necessary for HK-9. After 8 weeks *in vitro* cultivation the difference between HK-9H$_2$ and HK-9 was reduced to zero. Another passage through hamster liver restored the difference. HB-301 and HB-301H$_2$ both agglutinated at a con A concentration of approximately 20 µg ml^{-1}.

Antigenicity was analysed by immunoelectrophoresis (IEP) using human and rabbit anti-*E. histolytica* sera, and gel chromatography on Ultrogel AcA 34 and AcA 44 (LKB). The IEP pattern of HK-9H$_2$ showed at least one more precipitation line on the cathodal side than that of HK-9. Gel chromatography revealed a greater absorbance at 280 nm in the fractions with a molecular weight between 150.000 and 400,000. This will be investigated further.

We conclude that an increase of virulence in an attenuated axenic strain is involved with some, more or less stable, characteristics, such as toxicity towards host cells, of the surface membrane and antigenic make-up. The stability of these characteristics may depend on the homogeneity of genotypes within the population of a strain, which are constantly submitted to selective forces. In particular, the degree of toxicity towards leukocytes seems to be a vital characteristic of a virulent strain. *In vitro* toxicity against guinea pig leukocytes provides a convenient, quantitative test, which may prove useful to assess the pathogenic potential of a strain. Treatment with cholesterol or epicholesterol may be a useful way to adapt a non-virulent strain to hamster liver passage.

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Failure of BCG to protect calves against *Babesia divergens* infection

The possible use of nonspecific immunity to control *Babesia* infections has not been much examined. Cattle treated with *Escherichia coli* lipopolysaccharide (1.25 μg per kg live weight) immediately before infection with *B. argentina* (=*B. bovis*) were slightly more resistant than controls[1]. An interferon inducer, a double stranded RNA, slightly delayed death in mice infected with *B. rodhaini*[2]. The question has been reopened by some work with *B. microti* and *B. rodhaini* in which mice injected intravenously (i.v.) with 2×10^7 *Mycobacterium bovis* strain BCG (Glaxo strain) became completely immune to intraperitoneal challenge with 10^6 *Babesia* parasites[3]. These remarkable results suggested that the method should be examined in domestic animals. We decided to assess it in calves and report here that BCG fails to protect against infection with *B. divergens*, the common cause of redwater in the UK.

In a preliminary trial two splenectomised Friesian bull calves were used; one was inoculated i.v. with 50 ml of BCG suspension (a fresh, wet preparation, Glaxo) containing an indicated 3 mg ml^{-1}. Thirty-four days later both calves were challenged by the i.v.. inoculation of 0.1 ml of a *B. divergens* stabilate (Eire 2 strain). Both calves became infected and reacted similarly. This result was disappointing but because the use of splenectomised calves might have affected the outcome a second experiment using intact animals was carried out.

Glaxo provided 300 ampoules of lyophilised BCG vaccine, each ampoule containing 10^8 viable units. We dissolved the contents of each ampoule in approximately 0.5 ml of phosphate-buffered saline (PBS), the ampoules were thoroughly rinsed with PBS and the final suspension measured 200 ml. This was divided into three equal parts each of which contained approximately 10^{10} viable units. Each aliquot was inoculated immediately i.v. into a calf (K81, K82 and K84); no adverse side effects were noticed. Four weeks later these calves, together with three control animals (K56, K625 and K628) were challenged i.v. with 3.1×10^{10} parasitised erythrocytes (*B. divergens*, strain Eire 2). All the calves became infected; their reactions are summarised in Table 1. There was no indication that inoculation with BCG had suppressed the multiplication of *B. divergens*.

Table 1 Summary of results of challenge with *B. divergens*

Calf no.	Maximum parasitaemia (per 1,000 RBC)	Duration of parasitaemia (d)	Maximum temperature (°C)	Minimum RBC count ($\times10^6$ mm^{-3})
K81 : BCG	5	3	40.4	6.7
K82 : BCG	98	6	40.3	3.9
K84 : BCG	5	4	40.0	5.6
K56	5	3	39.7	7.1
K625	5	2	40.3	7.2
K628	3	3	40.1	6.1

One of the BCG calves (K82) reacted noticeably more severely than the others. At necropsy no macroscopic abnormalities were noted in any of the calves.

Various reasons for this failure can be adduced: one possible explanation lies in the dose of BCG. We had calculated that to repeat the work in mice as closely as possible it would be necessary to give 10^{11} *M. bovis* organisms to each calf but it was clearly impracticable to administer more than 10^{10} organisms.

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Development of resistance to cytotoxicity during aflatoxin carcinogenesis

It is now some time since Haddow first suggested that an early stage in chemical carcinogenesis might involve the induction of cells resistant to the toxic action of the carcinogen[1]. Although it seems that many carcinogens also have acutely toxic properties, the relationship between these activities is largely unknown. We have investigated this relationship by using the mycotoxin aflatoxin B_1, and shown that resistance is linked with proliferation of specific hepatocytes.

Aflatoxin B_1 is a potent hepatotoxin and hepatocarcinogen in many animal species including the rat[2]. Feeding young adult male Fischer rats a diet containing a low concentration (4–5 p.p.m.) of aflatoxin B_1 for 6 weeks, followed by return to control diet, results in 100% incidence of hepatocarcinoma[3]. During the first 3 weeks of the 6-week feeding regime, histological examination of the liver indicates an acutely toxic response, accompanied by depressed nucleic acid synthesis[4]. If toxic feeding is stopped at this stage an insignificant incidence of neoplasia results. The acutely toxic action results in the death of a considerable proportion of the hepatocytes at the end of the 3 weeks. The second 3 weeks of the 6-weeks feeding regime, during which the carcinogenic response is initiated, is accompanied by a proliferation of both parenchymal and nonparenchymal cells[3,4]. Nucleic acid synthesis also recovers[4]. The cells which proliferate to replace those killed by the acutely toxic action of the first 3 weeks feeding do so in the presence of a continuing supply of the toxin, showing that they possess a mechanism which makes them resistant to the acutely toxic, but not to the carcinogenic, actions of aflatoxin. The diet-induced resistance of the rats to the acute toxicity of aflatoxin is shown by the results in Table 1. The LD_{50} is increased at least 2–3-fold. This increase in the LD_{50} is paralleled histologically, by resistance to the hepatonecrotic effect of the toxin.

The possibility that the resistance of the liver cells in the whole animal to the acutely toxic action of aflatoxin B_1 could also be demonstrated *in vitro* was examined. Hepatocytes were isolated, and subsequently cultured, from livers of control animals (BL8) and livers of animals which had received the aflatoxin-contaminated diet (BL7) for 6 weeks. Morphologically, BL7 and BL8 cells appeared similar, but when treated with aflatoxin, BL7 cells survived and BL8 cells became structurally disorganised and detached from the dish (Fig. 1). Thus the resistance to the acutely toxic action of aflatoxin B_1, which was observed in the hepatocytes of intact animals which had received the toxic diet for 6 weeks, was also displayed by cells isolated from those livers and subsequently maintained in culture *in vitro* for 4 d. The isolated cells were not dividing at this time. In further experiments, cells isolated from control animals (BL8) or animals fed the toxic diet (BL7) were cultured with the medium being changed at approximately weekly intervals. After several weeks, or much sooner in cells isolated from rats fed the toxic diet, certain of the cells in the cultures started dividing, and numerous individual colonies were formed. Colonies were removed from the dish by trypsinisation and the dispersed cells were reseeded to establish rapidly dividing epithelial cell lines from each of the original maintenance cultures (BL7L and BL8L). These cell lines were indistinguishable morphologically. After several passages, the sensitivity of the cells to aflatoxin B_1 was again determined. Resistance of the original hepatocytes in the case of the aflatoxin-fed rats was still present in the cell lines derived from them. These results demonstrate that the resistance to the acutely toxic action of aflatoxin B_1, once established in the cells, is stable. Although most BL8L cells were killed when treated with aflatoxin B_1, a few survived. Dishes were washed with fresh medium 3 d after treatment with the toxin to remove dead cells, and returned to the incubator. Several weeks later colonies were observed to be growing from these surviving cells. These cells were then subcultured, after becoming confluent, and were subsequently passaged several times. Their response to the addition of aflatoxin B_1 to the medium was then determined. Cells derived from the susceptible cell line (BL8L) were now as resistant to the acutely toxic action of aflatoxin B_1 as the BL7L cells which had survived treatment *in vivo*. It therefore seems that resistance to the acute toxicity of aflatoxin B_1 depends on the proliferation of certain hepatocytes with either an inherent resistance to aflatoxin toxicity or the capacity for the induction of that resistance by aflatoxin B_1, either in the whole animal or in culture.

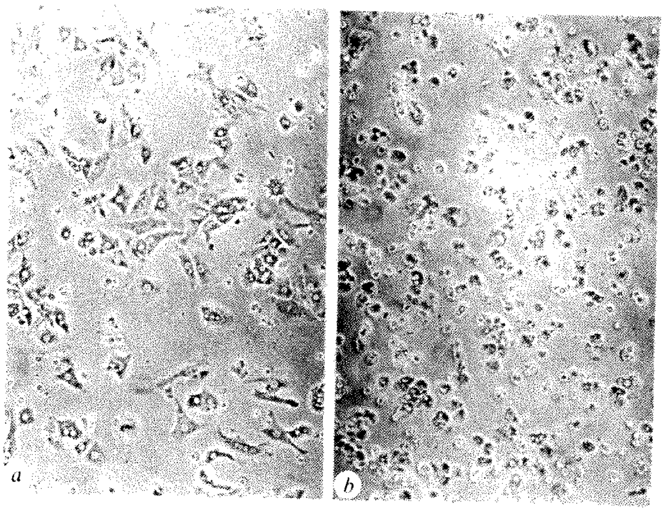

Fig. 1 Hepatocytes were isolated by perfusing liver excised from young adult male Fischer rats through the portal vein with collagenase solution (0.5% w/v) using a modification of the technique of Seglen[5]. The cellular suspension was further dissociated by agitation as described by Drockmans *et al.*[6]. Approximately 3 × 10^8 cells were obtained from each liver, with 80% viability assessed by exclusion of Trypan blue. Gross contamination was reduced by centrifugation twice at 50*g* for 2 min, and remaining cell clumps, broken cells and reticulo-endothelial cells were removed by sedimentation at 1*g* in a 1–4% Ficol gradient at 4 °C in a modification of the apparatus described by Tulp and Bont[7]. This process also yielded final cultures free of fibroblasts. 8 × 10^7 cells were layered on to a gradient. After collection by low-speed centrifugation, cells were suspended in Williams E medium containing 10% foetal calf serum (Flow Laboratories, Irvine, Scotland) at 1 × 10^6 cells per ml and 3 ml was seeded per 60 mm plastic Petri dish. Medium was changed after 1 d. After 3 d, medium was again changed and cells were treated with aflatoxin B_1 (0.3 μg ml^{-1} medium) in DMSO (6 μl ml^{-1} medium). Control dishes received DMSO alone. Appearance of cells after 2 d treatment with aflatoxin: *a*, cells isolated from rat which had received the toxic diet (see Table 1) for 6 weeks (BL7); *b*, cells isolated from control-fed rat (BL8). Cells receiving DMSO alone appeared as in (*a*).

Table 1 Effect of feeding a low level of aflatoxin B_1 on its acute toxicity

	Surviving after 7 d	
Aflatoxin B_1 injected (mg kg^{-1})	Control diet	Toxic diet
1.5	0	3
1.25	0	3
1.0	0	3
0.75	0	3
0.5	3	3
0.25	3	3
0	3	3

Control diet: 50% non-toxic peanut meal/50% powdered MRC 41 B diet plus 3.3% Arachis oil. Toxic diet: 25% toxic peanut meal (naturally contaminated with 16 ppm aflatoxin B_1), 25% non-toxic peanut meal, 50% powdered MRC 41B diet, 3.3% Arachis oil. Diets were fed for 6 weeks to young adult male Fischer rats (150–200 g body weight). Groups of three animals were injected with aflatoxin B_1 dissolved in dimethylsulphoxide (DMSO), all animals receiving a total injection volume of 200 μl. All deaths occurred within 3 d of the injections. No further deaths occurred until 3 weeks from time of injection, when the experiment was terminated.

The basis for the resistance is not known. It is not due to an overall failure of the cells to metabolise the toxin, for examination (thin-layer chromatography on silica gel G plates developed in chloroform–methanol, 9:1, (v/v, viewed under ultraviolet light) of a chloroform extract of the medium and cells, after incubating resistant cells (BL7L) in the presence of aflatoxin B_1 for 2 d, showed a considerable decrease in the concentration of residual aflatoxin B_1 compared with that found in control dishes which contained medium and aflatoxin B_1 but no cells. The resistance of the isolated cells and of the liver *in vivo* could be associated with a failure of the new cell population to metabolise aflatoxin B_1 specifically to an active metabolite. Such metabolism seems necessary for the inhibition of hepatic nucleic acid synthesis in *in vitro* systems[8], which correlates with the toxic effect of aflatoxin *in vivo*[4]. The results given in Table 2, however, show that neither the capacity of the microsomes to activate aflatoxin B_1, nor the susceptibility of nuclear RNA polymerase to the active metabolite, is affected by feeding the toxic diet. Other possibilities are that the resistance could be associated with the rapid repair of nuclear lesions, or with detoxification by other cellular factors. Our observation that the reduced glutathione content of the liver doubles as a result of 6

Table 2 Activation of aflatoxin B_1 *in vitro* to a form inhibitory to RNA polymerase *in vitro*

Source of fraction			RNA polymerase activity (d.p.m. incorporated per mg DNA)
Nuclei	Microsomes	NADPH	
CON	CON	–	55,500
CON	CON	+	19,200
CON	TOX	+	19,500
TOX	TOX	–	52,800
TOX	TOX	+	21,700
TOX	CON	+	19,700

TOX, animals fed toxic diet for 6 weeks (see Table 1): CON, animals fed control diet for 6 weeks. Assays carried out as described by Godoy and Neal[9]. Fractions were used in two successive incubations. First incubation, for activation of aflatoxin B_1: medium contained 50 μmol KCl, 150 μmol Tris-HCl *p*H 7.4, 12.5 μmol $MgCl_2$, 20 μl DMSO containing 10 μg aflatoxin B_1, 0.4 ml microsomal suspension (3.2 mg microsomal protein in 0.15 M KCl) and 1 ml nuclear suspension containing 150–200 μg DNA (in 0.44 M sucrose/1 mM $MgCl_2$). 0.75 μmol NADP, 10 μmol glucose-6-phosphate and 0.5U glucose-6-phosphate dehydrogenase were added to incubations as indicated. After 10 min incubation at 37 °C in air, nuclei were spun down at 4 °C and supernatant was decanted. Second incubation: Mg^{2+}-primed RNA polymerase activity of nuclei was determined as incorporation of label from ^3H–UTP during 10 min at 37 °C. Results are means of triplicate assays, variation < ± 10% of mean.

weeks of feeding with aflatoxin (unpublished) could be relevant to the detoxification process. •

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H–2 compatibility requirements for T suppressor cell functions induced by Friend leukaemia virus

GENE products of the major histocompatibility complex (H–2 in the mouse) regulate cellular interactions between various immunocompetent cell types[1–4] as well as interactions between thymus-dependent (T) killer cells and chemically altered or virus-infected target cells[5,6]. Identity of certain determinants of H–2 seems to be necessary for effective interaction between any two participating cell types. We have investigated the H–2 compatibility requirement for the suppressive interaction between T suppressor cells and mitogen-responsive cells in cultures infected with Friend leukaemia virus and find that there is an H–2D compatibiilty requirement. However, the H–2 restriction we observe is mediated by a third cell type which we term an 'interfering cell'. The two principal cell types, that is, T suppressor cells and mitogen-responsive cells, can interact even if they do not share any H–2 determinants provided that the interfering cells are functionally absent. This may provide an alternative explanation for the role the major histocompatibility complex in regulating other immune cellular interactions.

Friend leukaemia virus complex (FV) induces erythroleukaemia and causes severe immunosuppression in genetically susceptible adult mice[7]. FV mediates immunosuppression, at least in an *in vitro* model, by activating T suppressor cells[8,9]. The T suppressor cells prevent T and bursa equivalent-dependent (B) cells from responding to a variety of mitogens in the presence of FV. The population size and/or function of these T suppressor cells in the body are under the regulatory influence of marrow-dependent (M) cells, since treatment of genetically resistant C57BL/6 mice with ^{89}Sr to destroy M cells induces the generation or appearance of T suppressor cells. T suppressor cells are also present in thymuses of infant C57BL/6 mice before the maturation of M cells. Mixtures of spleen cells from normal C57BL/6 mice, which have no detectable T suppressor cells, with spleen cells from ^{89}Sr-treated C57BL/6 mice are suppressed by FV *in vitro*[9]. We have taken advantage of such a mixture protocol to determine the H–2 compatibility requirements between T suppressor cells and mitogen-responsive cells.

Spleen cell suspensions containing mitogen-responsive cells (but not suppressor cells) from 'resistant' B10.D2, B10.A, and C57BL/6 mice responded well to the mitogen, concanavalin A, in the presence of FV (Table 1). Addition of small numbers (10^5) of H–2-identical or semi-allogeneic 'susceptible' spleen cells, containing T suppressor cells, to the 9×10^5 spleen cells of 'resistant' mice resulted in suppression of mitogenesis by FV. The suppressor cells functioned after exposure to 800 R of γ rays *in vitro*.

However, suppression across the H–2 barrier was not effective. Adult DBA/2 (H–2^d) spleen, marrow or thymus cells failed to suppress C57BL/6 (H–2^b) spleen cells. The data of Table 1 and Fig. 1 clearly indicate that donors of cell suspensions containing T suppressor cells and the mitogen-responsive cells must share the same H–2D determinant for FV to induce immunosuppression *in vitro*. The critical experiment was the differential ability of 129 and DBA/2 spleen cells to suppress HTG lymphocytes (Fig. 1).

One explanation for the H–2 compatibility requirements for cytolytic T cells and target cells has been that T cells react to 'altered self'[5,6]. Indeed, antibodies directed against H–2D determinants inhibited cytolysis of syngeneic tumours by sensitised T cells[10,11], and an H–2D compatibility requirement for cytolytic T cells against Friend virus-induced tumours was observed[12]. An H–2D-end compatibility requirement for T suppressor cells has also been previously described[13]. In the AKR leukaemia model, T suppressor cells were restricted both by H–2 and non-H–2 differences because of an 'allogeneic effect'[14]. Another explanation for H–2 restriction has been the 'clonal selection' of T cells reactive to antigens presented on cells of a given H–2 type[15].

The H–2 compatibility requirement does not exist for

Fig. 1 H–2D compatibility requirements for mitogen-responsive cell-T suppressor cell interaction. See Table 1 for details of protocol. Open squares are H–2^d determinants; solid squares are H–2^b determinants; hatched squares are H–2^k determinants.

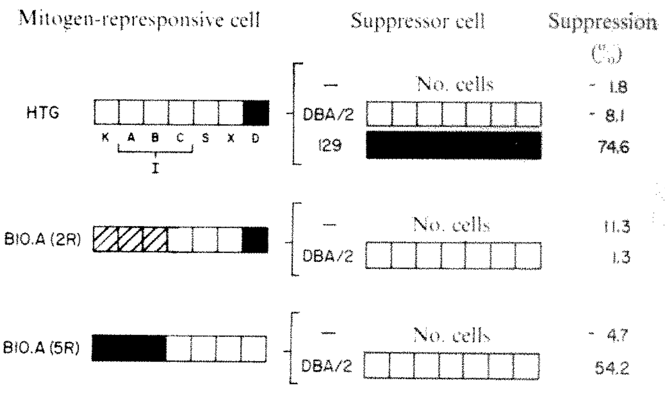

Table 1 H-2 compatibility requirements for Friend virus-induced suppression of mitogen-responsive cells by T suppressor cells

Experiment	Donor of spleen cell population: Resistant* (Mitogen-responsive cells)	Donor of spleen cell population: Susceptible* (T suppressor cells)	Change in Blastogenesis‡ −FV†	Change in Blastogenesis‡ +FV	Suppression %
1	B10.D2 (dddddd)§	None	62,573 ± 2,699	62,051 ± 2,714	0.8
		DBA/2 (dddddd)	60,234 ± 882	28,138 ± 1,713	53
		129 (bbbbbb)	49,314 ± 4,115	48,590 ± 497	1.4
		C3H (kkkkkk)	54,902 ± 692	57,863 ± 3,723	−5.0
2	B10.A (kkkddd)	None	49,971 ± 2,708	45,048 ± 1,581	9.8
		A (kkkddd)	50,150 ± 2,885	11,844 ± 468	76
		DBA/2 (dddddd)	36,143 ± 1,590	15,143 ± 1,590	58
		C3H (kkkkkk)	39,549 ± 915	34,840 ± 953	12
		129 (bbbbbb)	48,931 ± 1,815	45,904 ± 2,463	5.1
3	C57BL/6 (bbbbbb)	None	8,422 ± 655	7,229 ± 577	14
		B6D2F1 (bbbbbb/dddddd)	13,981 ± 8,255	6,762 ± 803	52
		B6D2F1 (800R *in vitro*)	14,988 ± 6,353	5,605 ± 931	63
		DBA/2 (dddddd) (spleen)	14,278 ± 1,992	11,960 ± 670	16
		DBA/2 (thymus)	16,060 ± 210	16,530 ± 5,110	−3.0
		DBA/2 (marrow)	35,637 ± 2,282	30,298 ± 4,931	15

*Resistant and susceptible to the leukaemic and immunosuppressive effects of Friend virus[7–9]. 9 × 10^5 resistant and 1 × 10^5 susceptible cells per well. Thymocytes and marrow cells were used in experiment 3 as indicated. Cells were cultured in triplicate in 0.2 ml volume in RMPI 1640 medium containing 10% foetal calf serum, with or without 0.5 μg concanavalin A (Con A) per well, for 72 h in Falcon Microtest II plates. ^{3}H-thymidine (0.5 μCi per well) was added 18 h before harvesting cultures with an automatic device[8,9]. B6D2F1 spleen cells were irradiated with a ^{137}Cs source just before culture in experiment 2.

†300 Focus-forming units of NB-tropic Friend virus complex per well.

‡Represents c.p.m. ^{3}H-thymidine incorporation, Con A minus no Con A, mean ± s.e.m.

§The lower case refers to the H-2 determinants K, IA, IB, IC, S and D.

cellular cooperation in antibody responses[16–18] or in T cell-mediated lysis[19] when the cell donors are tolerant to the antigens of the target cells. Even H-2-less cells can be lysed if concanavalin A is added to the reaction mixture, presumably to overcome a deficiency in 'recognition' of the target cell by the killer T cell[20]. In appropriate conditions *in vivo*, that is, transfer of 'resistant' marrow cells into irradiated 'susceptible' infant mice previously infected with FV, we had not observed any H-2 restriction for immunosuppression in the FV model[21]. Therefore, we considered the possibility that a third cell type, other than the T suppressor cell or the mitogen-responsive cell, was responsible for the H-2 restriction. If that were true, one may be able to overcome the H-2D compatibility requirement by treatments *in vitro* and *in vivo* which would eliminate the interfering cell.

We were able to overcome the H-2D restriction simply by irradiating the cell suspension containing T suppressor cells (Table 2). Moreover, spleen cells from an unhealthy DBA/2 donor mouse with a tiny thymus were capable of suppressing C57BL/6 mitogen-responsive cells. This alerted us to the possibility that adrenal steroids may remove the interfering cell, since steroids do cause severe hypoplasia of the thymus. Indeed, spleen cells from DBA/2 mice injected 2 d earlier with 2.5 mg cortisol were able to suppress mitogen-responsive cells of C57BL/6 mice in the presence of FV. Spleen cells from infant DBA/2 donors also suppressed H-2-incompatible C57BL/6 cells. To complete the analysis, we were able to 'reconstitute' the H-2 compatibility requirement by mixing adult untreated DBA/2 spleen cells containing the interfering cell with DBA/2 spleen cells devoid of such cells (Table 2).

We considered the possibility that an 'allogeneic effect' produced by interaction between interfering cells and H-2D-incompatible mitogen-responsive cells[14] might have been responsible for these findings. However, semi-allogeneic F1 hybrid spleen cells from susceptible donors were able to suppress parental-strain spleen cells from resistant donors (Table 1). This result indicates that allogeneic effect could not be responsible for failure of H-2-incompatible cells to interact in this system.

The complex immune cellular interactions so far detected

Table 2 Effect of source of DBA/2 spleen cell suspension on suppression of C57BL/6 mitogen-responsive cells

Experiment	Nature of DBA/2 spleen cell suspension*	Change in Blastogenesis‡ −FV†	Change in Blastogenesis‡ +FV	Suppression %
1	—	54,693 ± 9,889	54,513 ± 9,260	0.32
	Adult, 0 R	53,584 ± 9,526	54,165 ± 7,399	−1.0
	Adult, 1000 R	55,677 ± 1,292	32,192 ± 3,219	42
	1:1 0 R:1000 R	51,473 ± 2,450	55,541 ± 1,187	−7.9
	Infant	48,804 ± 3,926	33,629 ± 813	31
	1:1 Adult, 0 R:Infant	41,800 ± 1,757	53,982 ± 3,911	−29
2	—	58,358 ± 1,845	63,092 ± 3,354	−7.0
	Adult, Vehicle	53,830 ± 6,139	59,082 ± 1,789	−9.0
	Adult, Cortisol	34,874 ± 2,171	13,569 ± 2,001	61
	1:1 Vehicle:Cortisol	41,794 ± 5,849	40,720 ± 4,853	2.5

*Adult, 12 weeks of age; infant, 3 d of age. Spleen cells were exposed to 1000 R of ^{137}Cs γ rays *in vitro* just before initiation of culture. 10^5 DBA/2 cells were added to 9 × 10^5 C57BL/6 spleen cells from adult mice with con A and/or FV as described in legend for Table 1. Adult DBA/2 mice were injected with 2.5 mg cortisol (or saline vehicle) 2 d before the spleen cells were collected. DBA/2 mice are susceptible to FV and were the source of T suppressor cells.

†,‡See Table 1.

in the Friend leukaemia virus model are depicted in Fig. 2 and are summarised as follows. Marrow-dependent cells of genetically resistant mice, but not of genetically susceptible mice, restrict the numbers and/or functions of T suppressor cells *in vivo*[8,9] (Fig. 2*a* and *b*). T suppressor cells, even in the presence of syngeneic interfering cells, inhibit the proliferation of H-2D-identical mitogen-responsive cells in cultures infected with FV (Fig. 2*c*). Interfering cells, syngeneic with the T suppressor cells, prevent the suppression of H-2-incompatible mitogen-responsive cells (Fig. 2*d*). T suppressor cells, in the absence of syngeneic interfering cells, are quite capable of inhibiting the proliferation of H-2D-incompatible mitogen-responsive cells (Fig. 2*e*). Finally, mitogen-responsive cells of genetically resistant or susceptible mice proliferate well when exposed to FV, if no T suppressor cells are present in the mixture[8,9].

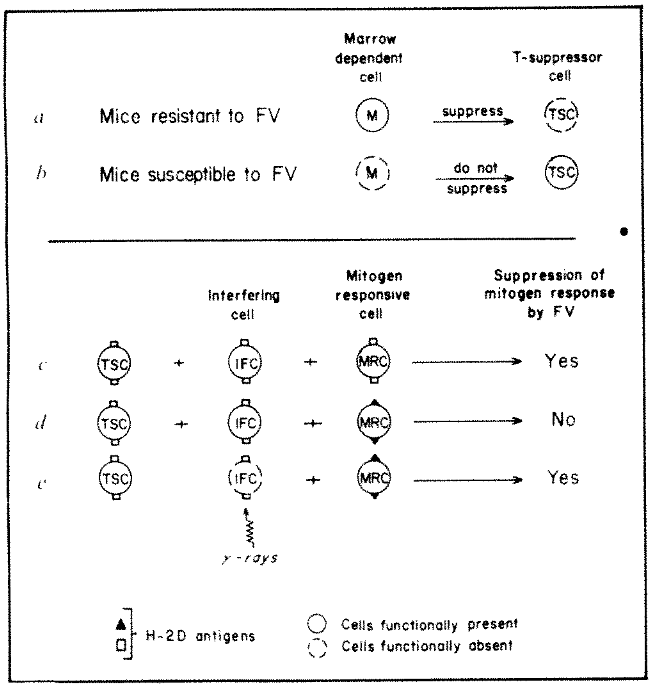

Fig. 2 Immune cellular interactions in the Friend leukaemia virus model. *a*, *b*: *in vivo*. *c*, *d* and *e*: *in vitro* cultures containing con A and FV.

One does not have to invoke 'altered self' or 'dual recognition' hypothesis to explain the H-2 compatibility requirements, at least in this instance. It is conceivable that the use of 'tolerant' cell populations to overcome the H-2 compatibility requirements in other studies[16-19] could be explained by the elimination of an interfering third cell. The mechanism of interference by the third cell type has yet to be determined, but the release of receptors for alloantigens[22] is a hypothesis worth testing.

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Sister chromatid exchanges induced by mutagenic carcinogens in normal and xeroderma pigmentosum cells

XERODERMA pigmentosum (XP) is a human disease characterised by sensitivity to sunlight[1], a high incidence of skin cancer[1] and a deficiency in DNA repair[2]. Investigations of DNA damage and repair in XP cells exposed to chemical carcinogens[3-8] had led to the classification of these agents in two groups: those in which damage can be repaired by apparently normal levels of excision repair (for example, monofunctional alkylating agents) and those in which it cannot (for example, 4-nitroquinoline-1-oxide) (Table 1). In XP cells, normal excision of MMS[9] or MNNG[10] damage is correlated with normal levels of chromosome aberrations, and reduced excision repair of 4NQO damage is correlated with high levels of chromosome aberrations[9], indicating that unrepaired damage is correlated with high levels of aberrations. It has recently been found that although sister chromatid exchanges (SCEs) differ from aberrations in many ways, they are far more sensitive indicators of chromosomal damage[11]. We therefore considered that a comparison of excision repairability with the yields of SCEs induced by chemicals might be informative in elucidating mechanisms of SCE formation. The results indicate that, contrary to the results with aberrations, the frequency of induced SCEs is higher in XP cells than in normal cells whether or not XP cells can perform normal amounts of excision repair of the chemically-induced damage. The data indicate that the lesions and/or subsequent events that give rise to chromosome aberrations are different from those leading to sister chromatid exchange and that SCE formation in XP cells

Table 1 Relative levels of excision repair (damage excision, unscheduled synthesis, or repair replication)* in xeroderma pigmentosum cells damaged by various chemicals or ultraviolet light

Mutagen	Level of excision repair in XP cells (group A) (% of normal)	Reference
Ultraviolet light	<5%	2
4-nitroquinoline-1-oxide	<5%	4,6,7
Mitomycin C	reduced	20
Dimethyl sulphate	100%	30
Methyl methane sulphonate	100%	3,8
Ethyl methane sulphonate	100%	8,30
N-methyl-*N*-nitro-nitrosoguanidine	100%	3,6,7
Ethyl nitrosourea	100%	30

*These measurements of repair replication and unscheduled synthesis were all made during the first hours after exposure and represent measurement of the more rapidly repaired damage.

is the most sensitive mammalian system yet described for detecting possible genetic effects of chemicals.

Human fibroblasts from a normal donor (GM637, Human Genetics Cell Bank, Institute for Medical Research, Camden, New Jersey) and from an excision-repair deficient xeroderma pigmentosum patient (XP12RO, Complementation Group A, donated by A. Veldhuisen, TNO Rijswick, The Netherlands) were treated with various concentrations of the chosen chemicals.

So that we could see sister chromatid exchanges, the cells were then grown for two rounds of DNA replication in the presence of 5-bromodeoxyuridine (BUdR) to produce "harlequin chromosomes" (ref. 12) in which the sister chromatids stained differentially.

The control yields of SCEs in normal and XP12RO cells are similar to one another (Table 2) and fall within the range previously reported for many cell types[13–16], with the exception of the very high spontaneous yields in cells from patients with Bloom's syndrome[14]. The control yields in the present experiments, in which the cells were exposed to 20 μM BUdR, are somewhat higher than the yields found after exposure to 10 μM BUdR (ref. 15), which is consistent with the observation that some of the SCEs are caused by the BUdR itself[17]. Once exposed to the chemicals, however, XP12RO cells contain higher numbers of SCEs than normal cells. This occurs even at concentrations too low to have detectable effects on normal cells (Table 2). The difference between normal and XP cells is most striking when MNNG (Fig. 1) is used, but is clearly evident for all the chemicals (Table 2). Kato noted that MNNG did not increase SCEs in normal Chinese hamster cells[31].

Since XP cells cannot perform excision-repair of damage induced by 4NQO, the elevated levels of SCEs, as well as the elevated levels of chromosome aberrations[9,10], and increased cell killing[6] observed after 4NQO treatment can be correlated with unrepaired damage. Such correlations also exist for ultraviolet damaged XP cells[18,19].

The same correlation cannot be made with damage induced by MMS, EMS, ENU, DMS, and MNNG, however, because XP and normal cells seem to undergo similar amounts of excision repair after exposure to these agents (Table 1). Nor can the correlation be made with damage induced by MMC because XP cells, which have been reported to be defective in unscheduled synthesis[20], respond as repair proficient cells for the induction of chromosome aberrations[20].

If XP12 cells are defective only in excision repair, then

Table 2 Chemically-induced sister chromatid exchanges in xeroderma pigmentosum and normal cells

Treatment	Normal cells (GM637) No. of SCEs/No. of chromosomes	Normal cells (GM637) SCEs per chromosome	Xeroderma cells (XP12RO) No. of SCEs/No. of chromosomes	Xeroderma cells (XP12RO) SCEs per chromosome
Control	1369/4397	0.31	1785/4867	0.37
Ultraviolet-like				
4NQO				
4×10^{-10} M	744/2191	0.34	1764/2584	0.68
4×10^{-9} M	770/2156	0.36	2025/2271	0.89
X-ray-like				
MMS				
10^{-7} M	661/2141	0.31	1182/2106	0.56
10^{-6} M	710/2150	0.33	1256/2280	0.55
5×10^{-6} M	860/1981	0.43	1696/2161	0.78
EMS				
10^{-6} M	769/2299	0.33	1388/2459	0.56
10^{-5} M	797/2226	0.36	2399/2453	0.98
MNNG				
10^{-9} M	537/1726	0.31	1060/2313	0.46
10^{-8} M	875/2207	0.40	1494/2450	0.61
10^{-7} M	842/2057	0.41	4076/2519	1.62
10^{-6} M	1381/2136	0.65	Too numerous to be scored	
ENU				
10^{-5} M	773/2061	0.38	829/1734	0.48
10^{-4} M	1042/1932	0.54	2222/2231	1.00
DMS				
10^{-6} M	771/1917	0.40	978/2016	0.49
10^{-5} M	809/1824	0.44	1457/1977	0.74
5×10^{-5} M	1650/2367	0.70	2872/2153	1.33
10^{-4} M	2823/2286	1.23	4111/2222	1.85
MMC				
10^{-10} M	612/1621	0.38	994/2288	0.43
10^{-9} M	754/1793	0.42	1199/1965	0.61
5×10^{-9} M	1009/2074	0.49	2818/2320	1.21
10^{-8} M	1483/2232	0.66	4379/2357	1.86

The cell lines were both transformed by SV40 and were chosen because of their high rate of cell division; previous studies[19,28] have demonstrated that transformation does not alter the DNA repair characteristics of human fibroblasts, although it does slightly increase the spontaneous frequency of sister chromatid exchanges in various cell types (normal, galactosaemic, Lesch–Nyhan and XP)[15]. Cells were grown in 75-cm^2 plastic flasks containing Eagle's minimum essential medium (MEM) with Earle's salts and 15% foetal calf serum. Twenty-four hours after the cells were recultured, BUdR (final concentration 20 μM) and the carcinogens were added to the flasks. The cells were then grown for two rounds of DNA replication (48 h) so that both strands of one chromatid and one strand of its sister would contain BUdR. Once BUdR had been added, the cells were kept in total darkness except during handling procedures, which were carried out in subdued light from incandescent bulbs to minimise any effects caused by the photolysis of BUdR-containing DNA. The yields of SCEs under these conditions were no higher than those obtained under total darkness. The carcinogens used included ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), *N*-methyl-*N*-nitro-nitrosoguanidine (MNNG), ethyl nitrosourea (ENU), dimethyl sulphate (DMS), 4-nitro-quinoline-1-oxide (4NQO), and mitomycin C (MMC). These were dissolved in medium (EMS, MMS), in phosphate-buffered saline at *p*H 7.2 (MNNG, DMS) or at *p*H 6.0 (ENU), in ethanol (4NQO), or in water (MMC) and then diluted with MEM to obtain the final concentrations that were to be added to cells. Colcemid (final concentration 2×10^{-6} M) was added to each flask for the final 6 h of exposure of BUdR. The mitotic cells were then shaken off the flasks, centrifuged and treated with a hypotonic solution (0.075 M KCl) for 6 min to spread the chromosomes. The cells were centrifuged, and the pellet was fixed and washed twice in 3:1 methanol/acetic acid. The fixed cells were concentrated by centrifugation and dropped on to dry microslides. The slides were stained in 5 μg ml^{-1} of Hoechst 33258 in phosphate-buffered saline at *p*H 6.8 (ref. 29) and then washed in distilled water, thoroughly dried, and mounted with a drop of Sorensen's buffer (*p*H 6.8) and a coverslip. They were then exposed to light one and a half inches from a high pressure mercury lamp (625 J m^{-2} s^{-1}) for 45 s to 5 min to allow the photochemical reaction to take place. The coverslips were then removed and the slides washed in distilled water, drained and then stained in 4% Giemsa (Gurr's R66) in M/15 Sorensen's buffer at *p*H 6.8, dried, and mounted in DePeX.

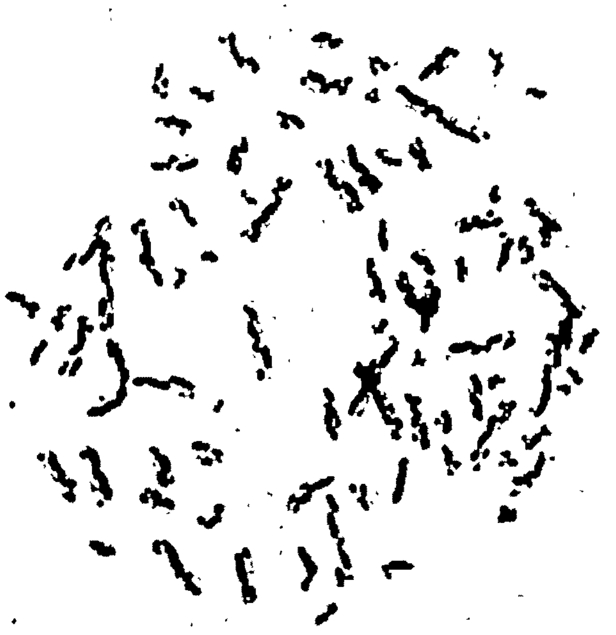

Fig. 1 A polyploid XP cell showing many SCEs after exposure to MNNG.

the increased yield of SCEs in such cells would be caused by unexcised damage in DNA. According to such a hypothesis, the results obtained with the alkylating agents would indicate that there is a minor fraction of the total damage remaining unexcised in each case. This fraction would be too small to be resolved by the usual measurements of repair replication or unscheduled synthesis, which have been made immediately after exposure to mutagens[3-6], as well as too small to give rise to higher than normal amounts of chromosome aberrations[9,10], or cell killing[8]. An increase in SCEs, however, is induced since they are very sensitive indicators of unexcised damage in DNA.

These observations, and the apparent lack of correlation between SCE frequencies and either chromosome aberration frequencies or cell killing (in the case of the alkylating agents) raise questions about the biological significance of SCE formation. SCEs differ in many respects from chromosome aberrations: SCEs are induced at high frequencies by chemicals at concentrations that induce few aberrations[11]; SCEs are not markedly increased by low doses of ionising radiations[21], whereas aberration frequency is; SCEs saturate with increasing doses of ionising radiation[22], and with tritiated thymidine[23]; SCEs react differently to treatments with caffeine than do chromosome abberrations[24,25]; SCEs are not correlated in any consistent fashion with the increased aberrations seen in the human diseases, Bloom's syndrome[14] ataxia telangiectasia[18] and Fanconi's anaemia[26], since SCE levels are very high in Bloom's, normal in ataxia telangiectasia, and reportedly low in Fanconi's anaemia.

SCEs may thus be the result of fundamentally different cellular events and lesions than are chromosome aberrations. Since the latter are associated with cell death, SCEs may be more representative of events compatible with cell survival, including mutagenesis. Although the majority of SCEs are probably genetically neutral because equal amounts of homologous chromatids are exchanged, some could conceivably be involved in deletion, insertion or frame shift mutagenesis because of a slight inequality of the exchanged material. Indeed, Magni and von Borstel[27] have found that mutation in yeast is correlated with meiotic exchange.

The sensitivity of XP cells we have observed indicates that whatever may be the genetic significance of SCEs, their measurement in XP cells is the most sensitive indicator yet found for detecting chromosomal effects of potential mutagens and carcinogens in mammalian cells.

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Drugs which disrupt microtubules do not inhibit the initiation of lymphocyte activation

LYMPHOCYTES are activated to grow and divide when mitogens such as plant lectins bind to surface receptors. Several hypotheses have been advanced to explain how a signal which results from the interaction of an activating ligand with its receptors, may be transduced across the membrane to initiate cell activation[1]. Recently Edelman and co-workers proposed a model in which a complex, composed of receptor, microfilaments and microtubules serves as a regulator for lymphocyte mitogenesis[2-4]. From experiments in which colchicine, when added to stimulated cultures at various time intervals, gradually lost its capacity to suppress subsequent DNA synthesis over 30–40 h, it was concluded that drugs which disrupt microtubules block lymphocyte activation at the point at which a lymphocyte becomes committed to undergoing growth and division[5,6]. However, other similar experiments[7,8] showed that colchicine was effective in suppressing DNA synthesis up to 48 h after the addition of mitogen. If indeed the microtubules are involved in the initiation of lymphocyte activation, then the disruption of these cytoplasmic structures should also affect cellular events which occur before DNA synthesis[9].

Here we report that two microtubule active drugs, colchicine and vinblastine, failed to affect events which occur early in stimulated lymphocytes, namely the increased RNA synthesis, the synthesis of a specific protein, lymphotoxin, and the increased turnover of membrane phospholipids. These results strongly suggest that microtubules are

Table 1 The effect of colchine or vinblastine on the concanavalin A-induced incorporation of ^{3}H-thymidine into DNA*

Concentration of drugs (M)	c.p.m. (10^{-3}) per 4 × 10^5 lymphocytes†	
	Colchicine	Vinblastine
Control	59.9±9.2	59.9±9.2
10^{-4}	15.7±1.0	0.6±0.9
10^{-5}	13.0±5.4	16.9±5.6
10^{-6}	18.3±0.9	17.7±5.1
10^{-7}	29.4±2.1	35.5±10.2
10^{-8}	39.8±7.9	52.3±3.5

* Incorporation into unstimulated lymphocytes: 0.6±0.2.

† Data given are means of triplicates ± s.d. 4 × 10^5 lymphocytes were cultured in microplates in 0.2 ml DME supplemented with 10% FCS in a water-saturated air–CO_2 atmosphere to maintain a *p*H of 7.2–7.4. Concanavalin A (2 μg) and the microtubule active drugs were added at the beginning of the experiment. After 54 h of culture 1μCi ^{3}H-thymidine (specific activity 1 Ci mmol^{-1}) was added and the lymphocytes were incubated for further 16 h. DNA was collected with an automated sample harvester and the radioactivity retained on the glass filters was measured by liquid scintillation counting.

not involved in the process which is responsible for the commitment of lymphocytes.

The experiments were performed with lymphocytes from the mesenteric lymph nodes of 8–12-month-old rabbits which were grown under SPF conditions. For measuring DNA synthesis, cells were cultured at 2×10^6 ml^{-1} in Dulbecco's modified Eagle's medium (DME) supplemented with 10% calf serum (FCS) in microtitre plates. To determine RNA synthesis, lymphocytes at 1×10^7 ml^{-1} were cultured under similar conditions. The incorporation of phospholipid precursors into lecithin was measured in lymphocytes, cultured at 10^7 cells ml^{-1} in DME supplemented with 2.5 mg ml^{-1} defatted albumin. Lymphotoxin was tested in supernatants from lymphocytes cultured at 10^7 ml^{-1} in DME+10% FCS for 24 h. In all experiments the microtubule active agents were added together with concanavalin A at the beginning of each experiment.

Table 1 shows that colchicine and vinblastine suppressed the incorporation of ^{3}H-thymidine into DNA of lymphocyte cultures stimulated with concanavalin A. Concentrations of 10^{-8} M and higher proved to be active. At concentrations of 10^{-5} the suppression of DNA synthesis ranged between 57 and 78% (mean 67%) with colchicine and between 63 and 82% (mean 72%) with vinblastine. In a concentration range up to 10^{-5} M both drugs were not toxic for lymphocytes after 4 and 20 h of culture, as assessed by the exclusion of trypan blue. At concentrations of 10^{-4} M, vinblastine started to be cytotoxic which was also found with colchicine at concentrations higher than 10^{-3} M.

The incorporation of ^{3}H-uridine into RNA becomes measurable early in the course of activation. From measurements taken at 4 or 22 h, neither drug, at concentrations up to 10^{-5} M influenced the uptake of ^{3}H-uridine into RNA of stimulated lymphocytes (Table 2). A remarkable suppression of ^{3}H-uridine uptake was, however, always observed in unstimulated lymphocytes after 22 h. These data would favour the notion that colchicine as well as vinblastine acts on the permeation of ^{3}H-uridine into the cell, which is overcome in stimulated lymphocytes.

Table 3 The effect of colchicine and vinblastine on the secretion of lymphotoxin

Concanavalin A	Colchicine (10^{-5} M)	Vinblastine (10^{-5} M)	Inhibition of cell growth (%)
—	—	—	0
—	+	—	9.5±0.5
—	—	+	10.3±0.5
+	—	—	20.4±1.0
+	+	—	25.3±1.3
+	—	+	28.9±1.5

Data are means of quintuplicates ± s.d. Lymphocytes were cultured at cell densities of 10^7 cells ml^{-1} in DME supplemented with 10% FCS. Con A (10 μg ml^{-1}) and microtubule-disrupting drugs were added at the beginning of the experiments. After 24 h the lymphocytes were removed by centrifugation for 10 min at 350*g* and the cell-free supernatants (containing lymphotoxin) were collected. As target cells for the action of lymphotoxin a permanent cell line F1 (derived from the human placenta) was used. Lymphotoxin activity was determined by the inhibitory effect on the ^{3}H-thymidine incorporation into the target cells.

Lymphocytes which are activated by plant lectins are able to mimic the immune functions which are physiologically expressed after antigenic stimulation. In T lymphocytes, which are activated by concanavalin A, this includes the synthesis of biologically active proteins, the lymphokines. The secretion of one of the lymphokines, namely lymphotoxin, which causes impairment of cell proliferation or cell death was therefore measured in supernatants of lymphocytes stimulated with concanavalin A. After a culture period of 24 h lymphotoxin was present in stimulated cultures but not in controls (Table 3). The addition of colchicine or vinblastine at concentrations of 10^{-5} M only marginally affected the production of lymphotoxin, when measured by its capacity to suppress cell growth. Control incubations cultured in the presence of the microtubule active drugs alone also caused some reduction of target cell proliferation which, however, was much less apparent than those caused by the lymphotoxin. The measurement of growth reduction through biologically active substances in the presence of drugs which themselves inhibit proliferation may be difficult to interpret. Therefore the direct lytic effect of lymphokines (measured

Table 2 The effect of colchicine or vinblastine on the incorporation of ^{3}H-uridine into RNA

Hours of culture	Concentration of the drugs (M)	c.p.m. (×10^{-3}) per 2 × 10^6 lymphocytes*			
		Colchicine		Vinblastine	
		Control	Con A	Control	Con A
	Control	1.67±0.18	3.03±0.51	1.67±0.18	3.03±0.51
	10^{-4}	1.40±0.09	2.50±0.12	0.83±0.04	0.83±0.50
4	10^{-5}	1.75±0.09	2.76±0.23	1.43±0.05	3.23±0.15
	10^{-6}	1.58±0.04	3.27±0.11	1.67±0.09	3.44±0.16
	Control	0.84±0.10	4.20±0.32	0.84±0.10	4.20±0.32
	10^{-4}	0.45±0.13	3.14±0.29	0.04±0.01	0.40±0.01
22	10^{-5}	0.60±0.02	4.17±0.14	0.42±0.03	4.00±0.11
	10^{-6}	0.64±0.10	4.36±0.71	0.66±0.02	4.40±0.25
	Control	0.87±0.15	5.20±0.86	0.87±0.15	5.20±0.86
22†	10^{-5}	0.55±0.04	5.00±0.78	0.54±0.16	5.20±1.05

* Data are given as means of triplicates ± s.d.

† Data given are means of three separate experiments ± s.d.

Lymphocytes (2 × 10^6) were incubated in 0.2 ml DME, supplemented with 10% FCS under conditions described in Table 1. After 2 or 20 h, 1 μCi ^{3}H-uridine (50 mCi mmol^{-1}) was added and the cells were cultured for additional 2 h. The cells were then collected as described in Table 1.

by the release of ^{51}Cr) was analysed with lymphotoxin preparations, after separation by chromatography on Sephadex G-150. The lytic effect of partially purified lymphotoxin from lectin-stimulated lymphocytes, which was eluted with 70,000–90,000 molecular weight substances, cultured with or without 10^{-5} M colchicine or vinblastine, was identical (18±5% after 4 h).

Very early in lectin-activated lymphocytes, the turnover of phospholipid fatty acids increased (10–12). Concentrations of 10^{-4} M of colchicine or of 10^{-5} M of vinblastine were unable to suppress the incorporation of ^{14}C-oleate into lecithin of con A-stimulated lymphocytes when measured after 4 h of incubation (Table 4). In four separate experiments, where colchicine or vinblastine were present at concentrations of 10^{-5} M, the incorporation of ^{14}C-oleate into lecithin of con A-stimulated lymphocytes reached 99±11 (colchicine) or 98±7 (vinblastine) percent of controls (data given with standard deviation). The turnover of the phospholipid fatty acids may also be followed by labelling the long chain fatty acids biosynthetically with ^{14}C-acetate[10]. As shown in Table 4, colchicine at a concentration of 10^{-4} M, or vinblastine at a concentration of 10^{-5} M caused no decrease of the ^{14}C-acetate uptake into lecithin of con A-stimulated lymphocytes. However, at concentrations higher than 10^{-5} M, vinblastine markedly decreased the incorporation of ^{14}C-acetate into lecithin of resting lymphocytes. These findings suggest that vinblastine in high concentrations primarily interferes with the permeation of acetate into lymphocytes.

Table 4 The effect of colchicine or vinblastine on the incorporation of ^{14}C-oleate or ^{14}C-acetate into lecithin*

	^{14}C-oleate mmol lecithin per 10^7 lymphocytes after 4 h culture†			
Concentration of the drugs (M)	Colchicine		Vinblastine	
	Control	Concanavalin A	Control	Concanavalin A
Control	0.61	1.27	0.61	1.27
10^{-3}	0.61	1.11	—	—
10^{-4}	0.81	1.29	0.37	0.43
10^{-5}	0.78	1.41	0.84	1.31
10^{-6}	0.79	1.36	0.71	1.39
	^{14}C-acetate c.p.m. (10^{-2}) in lecithin per 10^7 lymphocytes after 4 h of culture†			
	Colchicine		Vinblastine	
	Control	Concanavalin A	Control	Concanavalin A
Control	13.9	25.0	13.9	25.0
10^{-3}	9.3	16.9	—	—
10^{-4}	13.8	26.0	6.2	6.3
10^{-5}	12.2	29.3	6.9	24.5
10^{-6}	13.8	25.4	10.4	23.7
10^{-7}			13.7	23.9

* This fraction also contains smaller contaminants of phosphatidyl serine and phosphatidyl inositol.

† Data are means of duplicates.

Lymphocytes (10^7) were incubated at 37 °C in 1 ml HEPES-buffered DME supplemented with 2.5 mg defatted albumin.

^{14}C-oleate (10 nmol) or 25 nmol ^{14}C-acetate were added at the beginning of the experiment. After incubation the lipids were extracted, separated by thin-layer chromatography as described previously, and incorporation into lecithin was measured by liquid scintillation counting[10].

When lymphocytes are activated by mitogens as the lectin concanavalin A, a sequence of metabolic events is initiated which finally leads to cell division. Our results confirm earlier reports that colchicine or vinblastine at concentrations which disrupt the microtubular system suppress incorporation of ^{3}H-thymidine into lectin-stimulated lymphocytes. Addition of colchicine 30 h after the mitogen proved to suppress subsequent DNA synthesis to the same degree as when concanavalin A and colchicine were added together at the beginning of an experiment (data not presented). Cellular changes, which occur before the initiation of DNA synthesis were not affected in stimulated lymphocytes in the presence of colchicine or vinblastine. The incorporation of ^{3}H-uridine into stimulated lymphocytes was not suppressed at concentrations of 10^{-5} M. Similar results have been reported recently by Betel *et al.*[8]. Furthermore, the secretion of lymphotoxin which is induced in activated lymphocytes, was not decreased in a similar concentration range. In addition, cellular changes which are induced very rapidly after the binding of a mitogen to the surface receptors, that is, the increased turnover of the membrane phospholipids, were similarly not inhibited by concentrations of colchicine as high as 10^{-4} M or 10^{-5} M vinblastine. Thus our data are not compatible with the concept that microtubule-disruptive agents prevent the initiation of lymphocyte activation. It therefore seems unlikely that the putative association of receptor molecules with microtubules plays a large part in the signal transmission in concanavalin A-activated lymphocytes.

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Habitat values and endemicity in the vanishing rain forests of Sri Lanka

TROPICAL forests are among the most seriously threatened terrestrial environments, and while biologists and conservationists argue for preservation, the plunder continues virtually unabated[1]. In Sri Lanka (Ceylon), for example, the southern rain forests and montane forests contain a rich assemblage of endemic animals, but despite the protestations of the local conservationists, deforestation is rapidly destroying these habitats. One reason for govenment inaction in Sri Lanka, as elsewhere, may be the inability of conservationists to make an objective case for the value or uniqueness of these forests. In this paper, we propose some new approaches to this old problem.

Habitat value judgments must be quantitatively expressed if they are to seem credible in this technological age. The dilemma is that all estimates of quality or value are inextricably rooted in cultural, ethical and aesthetic assumptions, which are themselves value judgments. So-called objectivity in such matters is obviously impossible even though agreement is often achieved, at least within a culture. The logical and epistomological problems notwithstanding, we suggest in this paper some indices of habitat value that have the virtues of simplicity, flexibility and (to us, at least) intuitive appeal. The simplest and most general form of the index is

$$V_i = (Q_i/A_i)\,1{,}000 \qquad (1)$$

when A_i is area of the *i*th habitat and Q_i is the number if species in

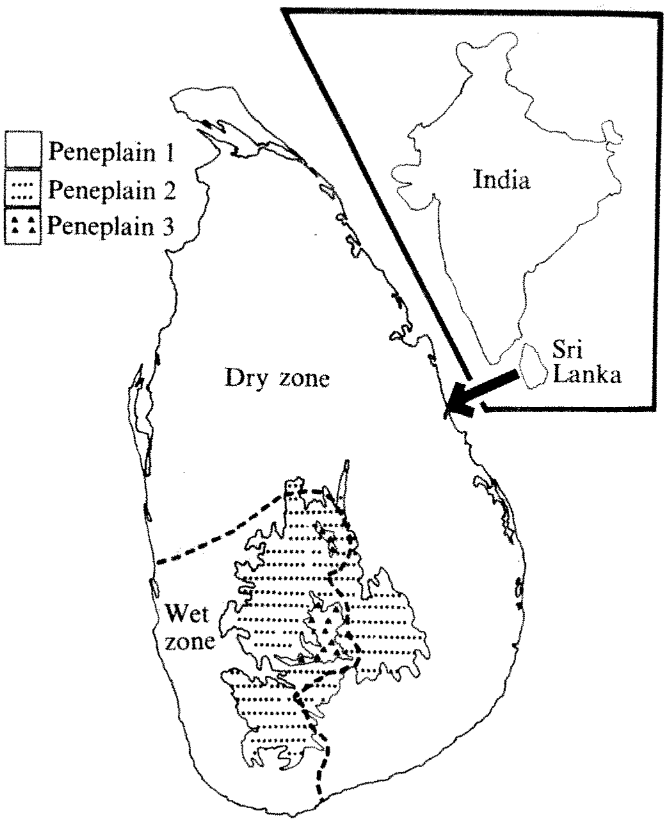


Fig. 1 The island of Sri Lanka showing the three peneplains and the dry and wet zones. The dashed line encloses the wet zone. The dry zone of peneplain 1 corresponds to region A; the wet zone of peneplain 1 to region B; peneplain 2 corresponds to region C; peneplain 3 corresponds to region D.

the *i*th habitat which have some quality deemed desirable. Depending on the location and purpose, "desirable" species could be those that are endemic, rare, large, or ancestors of cultivars, to list a few examples.

Under some circumstances, it would be appropriate to replace Q_i with a term that is the sum of desirability rankings. For example, endangered species occurring nowhere else might receive a rank of 10; an endangered species that occurs in the other habitats or regions might receive a rank of 5; rare species, but not officially endangered species, might receive a rank of 1. A similar but less arbitrary procedure is to employ the index

$$V'_i=\left(\Sigma\frac{1}{A_j}\right)1{,}000 \quad . \qquad (2)$$

when A_j is the area of the total geographic range of the *j*th species occurring in habitat *i*. This index has the advantage of being free of official judgments about the status of species and incorporating the commonest criterion for concern, namely, geographically restricted distribution. This approach would not be as appropriate as the ranking method for organisms, like some birds, which can simultaneously be both rare and widespread.

An important feature of index (1) (but not index (2)) is that the value of a habitat increases as the area it occupies decreases, even when there is no change in the number of species. Therefore, this index will be especially useful in comparing large areas with smaller, putatively more valuable ones. Conversely, it will not be very helpful when considering a large chunk of undifferentiated wilderness. Conservation arguments for the latter will often need to stress other considerations, including the space requirement of large, migratory species, the advisability of preserving large amounts of genetic variation in some species[2], and the needs of present and future generations for wilderness.

Figure 1 is a map of Sri Lanka showing the major biogeographical regions as defined by altitude, rainfall, and flora[3–8]. The island has been divided into three 'peneplains'. This term refers to well-marked plains of erosion. The lowest peneplain is by far the largest and surrounds the south-central hill country. The average elevation of this area is around 30 m, but hills within it reach 125 m. The second peneplain rises as a steep step from the first and averages around 400–500 m. The third peneplain is around 1,650 m.

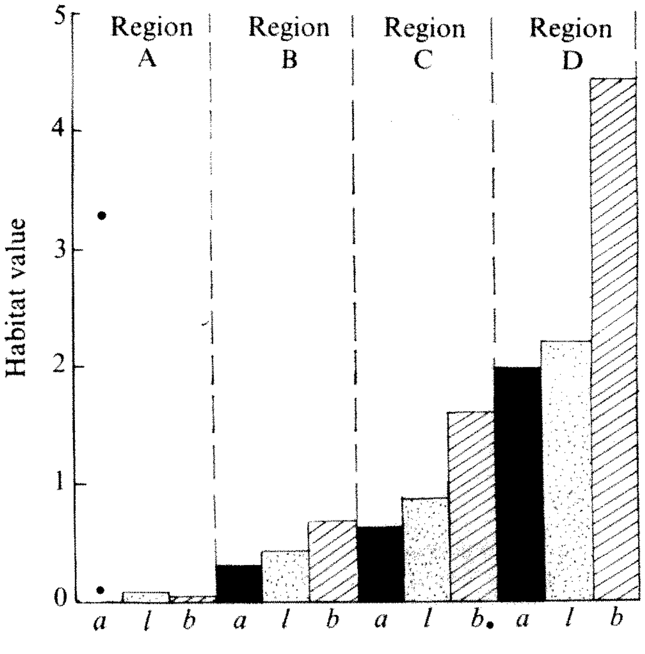
Precipitation is dependent on the trade winds and is seasonal. Two monsoons (rainy seasons) occur in Sri Lanka, the south-west (May to September) and the north-east (November to February). The rains of the south-west monsoon tend to be contained in the southern and south-western part of the island by the central mountain massif, while the north-east monsoon brings rains to both areas. As a consequence, two humidity 'zones' can be distinguished: the dry zone, that part of the island that receives only the north-east monsoon rains and an annual rainfall of under 190 cm; the wet zone which receives both monsoons and has an annual rainfall of over 190 cm. The second and third peneplains lie within the wet zone while the first peneplain is represented in both zones.

Based on the above geological and climatic zones, most authors have subdivided Sri Lanka into four biogeographic regions. We refer to these as follows: region A, the dry northern and eastern areas of the first peneplain or the dry zone; region B, the humid south-western part of the first peneplain; region C, the second peneplain; and region D, the third peneplain. Botanists have recognised floristic groupings that tend to reinforce this classification.

Recently, one of the authors (F.R.S.) spent 18 months on the island studying the distribution of reptiles and amphibians. The data indicate that the rain forest areas together with the montane regions in the south contain more endemic forms than any other part of the island. A similar pattern was observed in the avifauna. Cursory investigation of the insects and fishes seems to demonstrate a parallel distribution pattern.

Table 1 gives a list of endemic amphibians, lizards, and birds of Sri Lanka tabulated according to their occupation of the above defined regions. The methods on which the taxonomic decisions are based will be described elsewhere. We have chosen birds, lizards and amphibians as examples because information on their distributions is superior to that of other groups; for

Fig. 2 Habitat values (index (1)) of the four biogeographic regions in Sri Lanka: *a* indicates amphibia; *l* indicates lizards; *b* indicates birds.



example, Carl Gans, in the course of his work on the uropeltid snakes, is finding the published distributions for snakes to be very inaccurate. The available data[9–13] suggest that the patterns observed for the lizards, amphibians and birds ought to be reflected in the freshwater fishes and mammals.

In Table 2, we apply the habitat value indices to the Sri Lanka data; we consider only the species that occur nowhere else but on Sri Lanka. These endemics have considerable biogeographic and ecological significance because most of them are phylogenetic relicts whose closest relatives occur in South Africa, Madagascar, the Comoro Islands, Malaysia, and Sumatra. In employimg V_i' we should, strictly speaking, base our calculations on all the species on Sri Lanka, whether endemic or not. We chose not to do this for two reasons: first, the distributions of many forms is poorly

Table 1 Endemic amphibians lizards and birds of Sri Lanka

	Region			
Amphibians	A	B	C	D
Bufo kelartii			X	
B. atukoralei		X		
Rana greenii			X	X
R. corrugata		X	X	
R. gracilis		X		
Nannophrys guntheri		X		
N. ceylonensis			X	
N. marmorata				X
Rhacophorus cruciger		X	X	
R. eques				X
R. microtympanum				X
R. nasutus			X	
Philautus schmardanus				X
Ramenella obscura			X	X
R. palmata				X
Microhyla zeylanica				X
Lizards	A	B	C	D
Calotes calotes		X		
C. liolepis			X	
C. liocephalus				X
C. ceylonensis	X			
Ceratophora aspera			X	
C. stoddartii				X
C. tennentii				X
Cophotis zeylanica				X
Lireocephalus scutatus			X	
Otocryptis weigmanni		X		
Cnemaspis podihuna	X			
C. jerdonii			X	
Gymnodactylus frenatus			X	
Geckoella trierdrus				X
G. yakhuna			X	
Hemidactylus depressus	X			
Mabuya macularia		X		
Dasia haliana	X			
Sphenomorphus striatopunctatus				X
S. taprobens		X		
S. deigani			X	
S. dorsicatenatus			X	
S. fallax		X		
Chalcidoseps thwaitesi				X
Nessia butoni			X	
N. didactyla		X		
Evesia monodactyla				X
Bipedos sarasinorum		X		
B. smithi				X
Anguinicephalus layardii			X	
A. kickanale	X			
Birds	A	B	C	D
Cissa ornata (Ceylon Blue magpie)				X
Turdoides rufescens (Ceylon Rufous Babbler)		X	X	X
Turdoides cinereifrons (Ashy Headed Babbler)			X	X
Pycnonotus melanicterus (Black Capped Bul-Bul)	X	X	X	
Kelaartia penicillata (Yellow Eared Bul-Bul)				X
Oreocincla spiloptera (Spotted Winged Thrush)		X	X	X
Arrenga blighi (Ceylon Whistling Thrush)			X	X
Muscicapa sordida (Dusky Blue Flycatcher)				X
Bradypterus palliseri (Ceylon Warbler)			X	X
Eulabes ptilogenys (Ceylon Grackle)			X	X
Zosterops ceylonensis (Ceylon Hill White Eye)				X
Sturnia senex (White Headed Starling)			X	X
Dicaeum vincens (Legges Flower Pecker)		X	X	X
Cyanops flavifrons (Yellow Fronted Barbet)			X	X
Tockus gingalensis (Ceylon Grey Hornbill)		X	X	X
Phaenicophaeus pyrrhocephalus (Red Faced Malkoha)	X	X	X	
Centropus chlororhynchus (Green Billed Coucal)		X	X	
Psittacula calthorpae (Layards Parakeet)		X	X	X
Loriculus beryllinus (Ceylon Lorikeet)		X	X	
Columba torringtoni (Ceylon Wood Pigeon)			X	X
Gallus lafayettii (Ceylon Jungle Fowl)	X	X	X	X
Galloperdix bicalcarata (Ceylon Spur Fowl)		X	X	X

Table 2 Habitat value data and indices for the amphibians, lizards and birds of Sri Lanka

Region	Area (km²)	Endemic amphibians No.	Endemic amphibians V_i	Endemic amphibians V_i'	Endemic lizards No.	Endemic lizards V_i	Endemic lizards V_i'	Endemic birds No.	Endemic birds V_i	Endemic birds V_i'	Totals No.	Totals V_i	Totals V_i'
A	66,593	0	0	0	5	0.08	0.08	3	0.04	0.03	8	0.12	0.11
B	16,058	5	0.31	0.26	7	0.44	0.44	11	0.69	0.30	22	1.43	1.00
C	11,266	7	0.62	0.57	10	0.89	0.89	18	1.60	0.75	35	3.11	2.21
D	4,048	8	1.97	1.61	9	2.22	2.22	18	4.45	1.65	35	8.65	5.48

known; and second, the results would not be changed significantly because of the very small value of $1/A$ for non-endemic species relative to endemic species.

For the amphibians and lizards, the two indices give nearly identical results. For the birds, however, the V_i' values are relatively small because of the wider distributions of the birds compared to the amphibians and reptiles. In Fig. 2, we illustrate the V_i values for the regions described above. In words, the meaning of the figure is that the highlands are approximately 50 times more valuable than the lowlands because the area available is so small and the number of desirable (endemic) species in the highlands is so high. The pattern of distribution we have illustrated for the endemic species also holds true at the generic level. Of the nine endemic genera of lizards and amphibians, eight were confined to the moist or montane zones, while only one genus occurs in all zones. Current data[9,14,15] on the freshwater fishes and mammals indicate a similar trend at the generic level in these groups.

The data in Table 2 reinforce the opinion held by many biogeographers' that the southern forests and highlands of Sri Lanka are a unique biological treasure trove of evolutionary relicts[16]. The habitat values tell the story. Darlington[17] suggested that the number of relict forms on the island may be no more than occur in the southern part of India, but our recent studies on the reptilian and amphibian fauna and published data[9,10] seem to indicate that Sri Lanka does indeed have a much higher number of relict forms than continental south India. Birds may be an exception, however, because of their greater mobility and corresponding higher rates of gene flow and colonisation.

The affinities of the endemic genera of reptiles and amphibians (some have congeners in Malaysia and Sumatra, others have their closest relatives in Africa and Madagascar) are noteworthy in light of recent attempts to interpret biogeographic patterns in terms of continental drift[18]. While the majority of total faunal representation is markedly Indian in origin, the percentage representation of the Indian element is much lower in the montane areas than in the lowland dry zone. Cruz[15], in our opinion, rightly argues that the montane zones represent the most conservative elements of the Sri Lankan fauna, those that have been least influenced by recent invasions from the Indian mainland.

The habitat values of the wet, montane regions, while relatively high, are quite conservative because the percentage of land that remains forested in the wet, montane regions (B, C, and D) is only 9% compared to over 30% of the total area of region A. It should be emphasised that the paucity of endemics in the lowlands is in large part a consequence of periodic land bridge connections to South India in the Pleistocene and the episodic exchanges of lowland biotas.

Sri Lanka, like most tropical developing countries is today faced with rapid population growth and the problems associated with the cultivation of agriculturally marginal lands and the intensified exploitation of natural resources. The forests are now being cleared for the production of plywood and pulp. Extinction of many animals will inevitably occur because the ecological successional process that follows deforestation leads to the development of habitats unsuitable to most of the endemic forms (to be discussed more fully elsewhere). Even nationally accredited areas of unique habitat have recently been brought under the axe and plough[15]. Biologists, geographers and conservationists should accomplish what research they can there before it is too late.

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Immunosuppression by mouse sialylated α-foetoprotein

AFP is the dominant serum protein in early embryonic life. It is considered also to be a tumour-associated antigen because it appears in the sera of patients and animals with primary liver cancer[1]. The function of AFP in the foetus remains unknown. Tomasi *et al.*[2] have demonstrated that mouse AFP has an immunosuppressive effect on antibody synthesis both *in vivo* and *in vitro*. Similarly, human AFP has been reported to have immunosuppressive properties[3]. One of us (E.F.Z.) has shown that mouse AFP is heterogeneous, consisting of five components (Fp1–Fp5)[4], which differ in the amount of sialic acid attached to the molecule: Fp1 contains no detectable sialyl residues while Fp5 has four per molecule[5]. We have now demonstrated that the presence of sialic acid residues in the AFP molecule is essential for the immunosuppressive activities of this glycoprotein.

The individual components of AFP were isolated as described before[6]. Briefly, mouse amniotic fluid or foetal plasma from days 15.5–16.5 of gestation were used as sources of α-foetoprotein. Components Fp1 and Fp5, respectively, were purified by electrophoresis on long preparative polyacrylamide gels at low amperage for 65 h in the cold room. Wedges were removed, the locations of AFP components were determined by fluorescence with ultraviolet-activated acidified anilino-naphthalene-sulphonic acid reagent, and the wedges were aligned with the untreated gels. Portions of the untreated gels corresponding to Fp1 and Fp5 were cut out and eluted with 0.05 M Tris-0.1 M NaCl, pH 7.0. Fp1 and Fp5 components were used in tests for immunosuppressive properties because they have no residues and four residues, respectively, per molecule. Because of the possibility that Fp1, derived from amniotic fluid, might lack internal sugars, Fp5 was treated with neuraminidase. The glycoprotein lacking the terminal sialic acid, designated Fp1^{n}, should now have its penultimate sugars on the oligosaccharide. Fp1^{n} was separated from the neuraminidase-resistant Fp3 and as indicated later it migrated to the same position as that of Fp1.

The immunosuppressive activity of the components was determined in mouse splenic lymphocyte cultures in which the primary immune response to sheep erythrocytes was measured. Female BDF$_1$ mice, 10–12 weeks old (Jackson Laboratory) served as the source of splenic lymphocytes. Splenic cultures were prepared as described by Mishell and Dutton[7]. Spleen lymphocytes were cultured with sheep erythrocytes for 5 d in the presence or absence of AFP components. The number of antibody-forming cells to sheep erythrocytes on day 5 was determined by Jerne's plaquing technique[8]. As established by the Trypan blue dye exclusion test, AFP components were not cytotoxic for splenic lymphocytes. Figure 1 illustrates AFP components as identified before and after electrophoretic purification. Electrophoretic analysis of Fp5 after treatment with neuraminidase indicates that it now is very closely related or identical to the Fp1 component. Furthermore, no apparent contamination of each purified AFP component was observed.

Figure 2 shows precipitin analysis of the AFP components by double diffusion technique in agar gel. Goat antiserum prepared against components Fp1–Fp3 was tested against homologous and heterologous AFP components. A total identity band between the various AFP components is evident indicating that all AFP components possess a common structure. Mouse serum from adult animals did not show a precipitin band formation with anti-AFP antiserum indicating that our AFP preparations were not contaminated with other serum proteins.

The immunosuppressive effects of AFP components are shown in Table 1. Mouse amniotic fluid (MAF) and component Fp5 were significantly immunosuppressive: MAF (100 μg), $P < 0.05$; Fp5 (1 μg), $P < 0.0005$. Components Fp1 and Fp1^{n}, however, exerted no effect on antibody formation to sheep erythrocytes. In addition, the acrylamide gel eluate, from which these AFP components were isolated, showed no immunosuppressive properties. Normal serum obtained from adult mice (NMS) was quite immunosuppressive although it contained no AFP (Fig. 2). Apparently additional immunosuppressive factors are present in normal animal sera as demonstrated before[9].

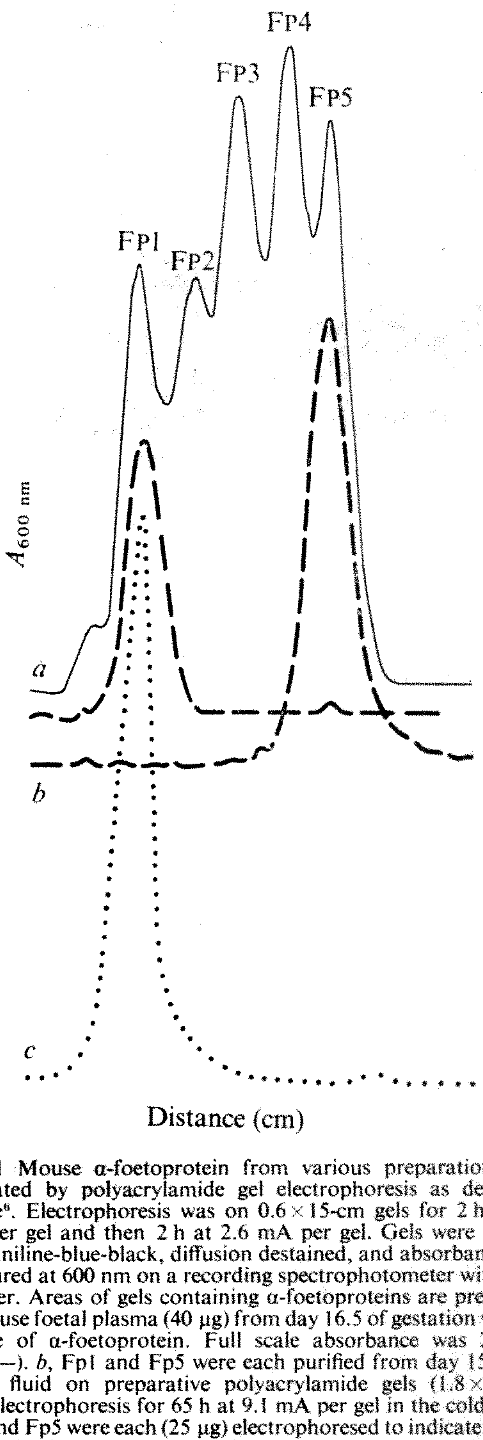

Fig. 1 Mouse α-foetoprotein from various preparations was separated by polyacrylamide gel electrophoresis as described before[6]. Electrophoresis was on 0.6 × 15-cm gels for 2 h at 3.5 mA per gel and then 2 h at 2.6 mA per gel. Gels were stained with aniline-blue-black, diffusion destained, and absorbance was measured at 600 nm on a recording spectrophotometer with a gel scanner. Areas of gels containing α-foetoproteins are presented. *a*, Mouse foetal plasma (40 μg) from day 16.5 of gestation was the source of α-foetoprotein. Full scale absorbance was 2 units (———). *b*, Fp1 and Fp5 were each purified from day 15.5 amniotic fluid on preparative polyacrylamide gels (1.8 × 44 cm) with electrophoresis for 65 h at 9.1 mA per gel in the cold room. Fp1 and Fp5 were each (25 μg) electrophoresed to indicate purity. Full scale absorbance was 3 units (— — —). *c*, Fp1^{n} was derived by treatment with *V. cholerae* neuraminidase from Fp5 purified from newborn plasma. Fp1^{n} was then separated from Fp3^{n} on a preparative gel, as described above, and Fp1^{n} electrophoresed on an analytical gel to indicate purity. Full scale absorbance was 3 units (·····).

Table 1 Effect of AFP components on the *in vitro* primary immune response to sheep erythrocytes

Preparation		PFC per 10^6 viable cells ± s.e.
Control		597 ± 135
Gel eluate		530 ± 132
MAF	(200 μg)	271 ± 82
MAF	(100 μg)	365 ± 368
MAF	(10 μg)	567 ± 8
Fp5	(10 μg)	284 ± 19
Fp5	(1 μg)	248 ± 96
Fp5	(0.1 μg)	506 ± 86
Fp1	(10 μg)	648 ± 168
Fp1	(1 μg)	467 ± 137
Fp1^{n}	(10 μg)	579 ± 228
Fp1^{n}	(1 μg)	709 ± 130
Fp1^{n}	(0.1 μg)	588 ± 134
NMS	(1%)	521 ± 150
NMS	(5%)	168 ± 38
NMS	(10%)	130 ± 104
NMS	(15%)	66 ± 11

The values were derived from at least two experiments, each of which contained triplicate determinations.

The apparent requirement for sialyl residues on the AFP molecule to achieve immunosuppressive properties parallels observations made in other biological systems. Remold and David have demonstrated[10] that macrophage inhibitory factor (MIF) which is produced by sensitised lymphocytes requires the presence of terminal sialyl residues for its inhibitory activity. It is conceivable that sialyl residues, which are quite often found on the surface of cellular membranes and serum proteins, may have an important role in the regulation of various biological properties. Because we used a primary lymphocyte culture for antibody

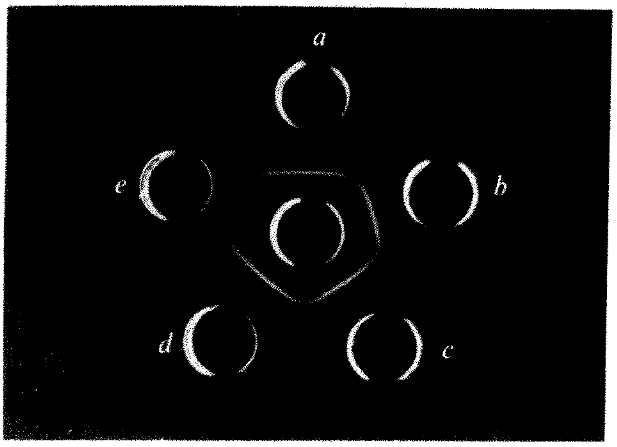

Fig. 2 Ouchterlony double immunodiffusion on an agar plate (Hyland) of α-foetoprotein preparations against anti-Fp1-3 (ref. 4). Source and purity of isolated α-foetoprotein preparations are indicated in Fig. 1. Outside wells contained: *a*, Fp5 (600 μg ml^{-1}); *b*, Fp1 (675 μg ml^{-1}); *c*, Fp1^{n} (550 μg ml^{-1}); *d*, day 14.75 mouse amniotic fluid (2.2 mg ml^{-1}), and *e*, mouse adult serum (undiluted). The centre well contained the antiserum.

formation to sheep erythrocytes, it can be assumed that in our experimental conditions IgM immune response was suppressed. Dattwyler *et al.*[11] have reported that AFP binds specifically to T lymphocytes and therefore this type of lymphocyte (or perhaps a subpopulation of T lymphocytes) may carry on its surface receptors for sialyl residues. The mechanism of immunosuppression by AFP or other serum factors remains unknown. It has been reported that immunosuppressive properties of mouse AFP are affected by binding of oestrogen[12]. It remains to be determined whether this binding is dependent on the presence of sialyl residues on AFP and whether the immunosuppression may involve more than one reactive substance. It is hoped that, as the structures in the lymphocyte membrane which can interact with sialyl residues are identified, the understanding of immunosuppressive processes will improve.

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Immunocytochemical studies of cells in the rat adenohypophysis containing both ACTH and FSH

STUDIES by Siperstein *et al.*[1,2] first described the cytology of the adrenocorticotropin (ACTH) cell in the rat adenohypophysis. This cell is stellate and contains peripherally organised granules averaging 220 μm in diameter. Their cytological work was confirmed in later immunocytochemical studies in our laboratory[3–5], in which ACTH was localised in the secretory granules of cells of this type. In our recent immunocytochemical studies of follicle-stimulating hormone (FSH) cells, however, FSH was found in a cell which resembled the ACTH cell. We termed this cell the Type III gonadotroph[6,7]. Here we describe studies designed to test the specificity of the reaction for FSH in this cell type, and the relationship between the Type III gonadotroph and the cell containing ACTH.

The immunocytochemical technique used was the unlabelled antibody peroxidase–antiperoxidase complex (PAP) technique of Sternberger *et al.*[8], as modified in our laboratory for the ultrastructural localisation of ACTH[3,4]. Pituitaries were fixed in picric acid formaldehyde and embedded in Araldite 6005. Ultrathin serial sections were mounted on nickel grids, etched in 10% H_2O_2 for 5–10 min, and then stained immunocytochemically.

The specificities of the immunocytochemical stains were determined by the absorption of each antiserum with ACTH or FSH for 2 d before its use in the stain. Table 1 lists the results of specificity tests of the anti-ACTH$_{17-39}$ sera and anti-rat FSHβ sera. Only previous absorption with ACTH abolished staining in the ACTH cell with the anti-ACTH$_{17-39}$ serum; previous absorption with FSH had no effect. Similarly, FSH absorption abolished FSH staining in the Type III gonadotroph; however, absorption with ACTH had no effect.

Other specificity tests included the substitution of antiserum to luteinising hormone (LH) or thyroid-stimulating hormone (TSH) for the anti-FSH or anti-ACTH sera. Cells resembling the Type III gonadotroph were not abundant in either the LH cell population[6,7] or the TSH cell population in normal male rats or cycling female rats. The omission of the goat anti-rabbit IgG from the staining sequence abolished staining completely.

After proof of specific staining was established, the next phase of the study was designed to test if the ACTH cell and the Type III cell were the same cell. For this purpose, the stains for ACTH$_{17-39}$ and FSHβ were used on serial sections of oestrous female rats (1300 h on day of oestrous), which have an abundance of Type III gonadotrophs. Twenty serial cells were collected and analysed for their hormone content. Of the group of cells collected, nine stained for ACTH alone (Fig. 1). The other eleven cells stained for both FSHβ and ACTH (Figs 1 and 2). Out of the cells analysed, we have yet to find a Type III gonadotroph which contains only FSHβ.

The insets and arrows in Fig. 2 indicate that we have serially sectioned some of the granules. An analysis of the staining pattern of each of 26 granules in 5 areas of the cell depicted in Fig. 2 shows that 12 contain only ACTH, 8 contain both ACTH and FSH, and 6 are negative. No granules were found which stain for FSH only.

In summary, it is evident that one of the FSH cell types is a cell morphologically identical to the ACTH cell, and the serial sections collected thus far indicate that this cell contains ACTH as well. Our findings are difficult to explain. The staining for FSHβ in a stellate cell can be explained from the standpoint of morphological differentiation. For example, in a particular physiological state, such as enhanced secretion, FSH gonadotrophs may become stellate following stimulation as an aid or adjunct to their activity. The finding that these cells contain ACTH as well is however, somewhat surprising since there is no known chemical homology between ACTH and FSH.

Sectioning 220–250-μm granules serially is difficult, and we were fortunate to obtain such a serial for the cell in Fig. 2. Our results show that when FSH staining is exhibited by a granule, it is found in a granule which also contains ACTH. If this staining simply reflected a subpopulation of antibodies to ACTH found in the anti-FSH serum, then one might expect that ACTH absorption would abolish it. The results in Table 1 show that ACTH absorp-

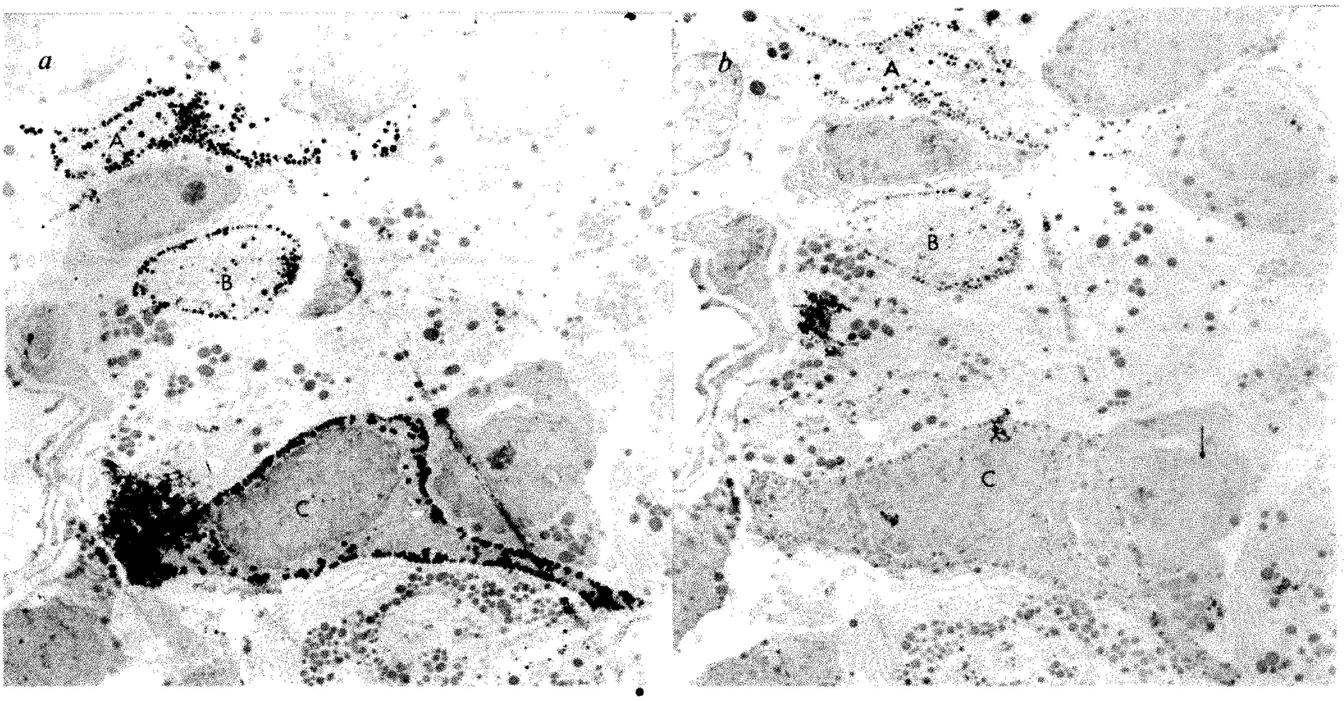

Fig. 1 *a*, Ultrathin section of rat pituitary stained immunocytochemically with 1:5000 dilution anti-ACTH$_{17-39}$ serum (48 h, 4 °C) followed by 1:100 dilution goat anti-rabbit IgG, (3 min) followed by 1:100 dilution PAP solution (3 min) and the histochemical reaction for peroxidase 3, 4. *b*, Section serial to that in *a*, stained with 1:2000 dilution anti-rat FSHβ followed by the same sequence of solutions as in *a*. Cell A stains intensely for ACTH and weakly for FSHβ. Cells B and C stain intensely for ACTH alone. Cell A resembles a gonadotroph (Type II)[7]. Only a few granules in Cell B stain for FSHβ; cell C clearly does not react with anti-FSHβ. Fixation: picric acid formaldehyde; embedded in Araldite 6005. Magnification: ×4500.

tion had no significant effect on anti-FSH staining, however. Therefore, this suggests that the staining reflects the presence of FSH or a molecule with some amino acid sequences identical to those in FSH.

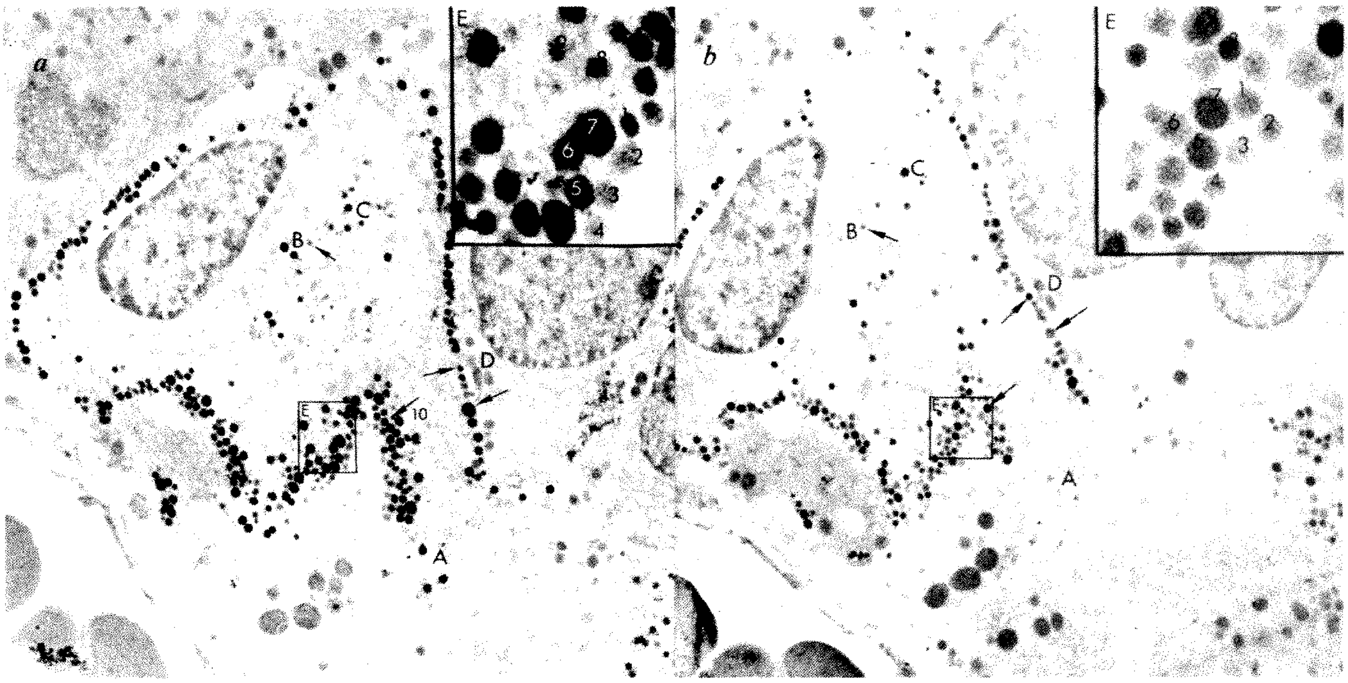

Fig. 2 *a*, Ultrathin section of female rat pituitary stained, immunocytochemically, with anti-ACTH$_{17-39}$ serum. *b*, Section serial to that in *a*, stained with anti-rat FSHβ. Cell A is stained with both antisera, although stain is weaker with anti FSHβ serum. The areas designated contain some of the same granules. Areas A and B show staining for ACTH on five granules. In area A of the cell stained for FSHβ, granules are faintly visible. No stain is evident on them, nor on those in Area B. One granule in Area B stains for neither ACTH or FSH (arrow). Area C contains two granules which stain for ACTH and FSHβ. Area D contains only one granule which stains for both hormones (arrow). The granule below it stains only for ACTH, as does the largest granule in the area (arrow). Area E was blown up as the inset. Granules 5, 7, 8, 9, and 10 stain for FSHβ and ACTH. Granules 1 and 6 stain only for ACTH. Granules 2, 3, and 4 do not seem to contain either hormone. Granule 10 is not shown in the inset in *a*; it is designated by an arrow in *a* and *b*. Magnifications: ×4500, inset ×20,000.

Table 1 Densitometric analysis of specificity of the reaction with the anti-FSHβ and the anti-ACTH sera

Antiserum*	Normalised staining intensity† of secretory granules
Anti-FSHβ‡	0.20±0.002 s.e.m.
Anti-FSHβ + 1 ng FSH ml^{-1}	0.15±0.01
Anti-FSHβ + 10 ng FSH ml^{-1}	0
Anti-FSHβ + 10 μg ACTH ml^{-1}	0.18±0.02
Anti-ACTH$_{17-39}$ sera§	0.30±0.01
Anti-ACTH$_{17-39}$ sera + 10 ng ACTH ml^{-1}	0.18±0.008
Anti-ACTH$_{17-39}$ sera + 100 ng ACTH ml^{-1}	0
Anti-ACTH$_{17-39}$ sera + 1 μg FSH ml^{-1}	0.32±0.13

*1:2000 dilution anti FSHβ, 1:5000 dilution anti-ACTH$_{17-39}$. Antigen added to 1 ml of diluted sera 2 d before its use in the stain.

†Normalised staining intensity values are optical density measurements of 50–75 granules per sample. Nuclear chromatin used as background to correct for section thickness 4.

‡Obtained from Dr A. F. Parlow, National Institutes Arthritic Digestive and Metabolic Diseases. FSH antigen-I-3 preparation.

§Produced in this laboratory. ACTH antigen-III World Standard.

There is little evidence for a functional relationship between FSH and ACTH. There is evidence for adrenal control of gonadal cycles[9] and oestrogen stimulation of ACTH release[10,11]. As discussed in a recent review[9], however, there is more evidence for a functional link between ACTH and LH than ACTH and FSH. Yet our studies show that Type III LH cells are infrequent. They comprise only 4–7% of the total LH cell population, compared with 20–30% of the FSH cell population in cycling female rats[7].

Recent studies by Pouplard *et al.*[12] have shown that human pituitary ACTH cells bind Fc fragments in a manner similar to that exhibited by mast cells and basophils. It is possible that the staining in rat ACTH cells represents a similar nonspecific affinity for immunoglobulin molecules. If this were the case, however, the controls in our study would have shown staining; for example, we would not have abolished staining by previous absorption with the antigen. Therefore, our tests show that the staining is more likely to be a specific immunochemical reaction for FSH and ACTH.

In conclusion, it seems that the old system of identification of pituitary cell types (based on granule size, distribution, cell shape) does not provide accurate information about a cell's hormone content. This is certainly true for the gonadotrophs[6,7], and as we have shown in this study, it is true for ACTH cells as well. One cannot distinguish an ACTH cell from an ACTH–FSH cell solely on the basis of morphology. We recognise the need for studies of animals in different physiological states before further conclusions are drawn. In fact, the relationship between the two hormones may become clearer as more animals in different physiological states are examined. We also recognise the need for further purification of the antisera.

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Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat

THE isolation and identification of endogenous opiate-like peptides from mammalian brain[1,2], and from pituitary[3–5] has led to an active investigation of their pharmacological properties. These peptides—the enkephalins and endorphins—have been shown to produce naloxone-reversible analgesia[6,7], tolerance, and withdrawal[7]. While these effects seem consonant with those of morphine and other exogenous narcotics, little is yet known about the intrinsic, physiological functions of the opioid peptides. Since powerful analgesia is a prominent and reliable phenomenon associated with the administration of both narcotic alkaloids and brain opioid peptides, it seems reasonable to suggest that the opioid peptides may normally function to modulate pain responsiveness. Furthermore, work with stimulation-produced analgesia has suggested the existence, in mammalian brain, of a pain-inhibitory system with an opiate-like link[8,9]. We have, therefore, hypothesised that such an endogenous system may be engaged by either environmental, sensory, or other physiological events which lead to adaptive changes in pain responsiveness. For example, noxious or stressful stimuli may recruit pain-inhibitory mechanisms in the central nervous system, bringing about alterations in enkephalins and endorphins, with concurrent or subsequent changes in responses to pain. Consistent with this hypothesis, we now report that inescapable acute stress causes a significant increase in levels of opioid peptides with a concurrent decrease in pain responsiveness in the rat. Furthermore, both the biochemical and behavioural changes are "reversed" by repeated exposure to stress. A preliminary report of this work has been made[10].

To assess the effects of either acute or repeated exposure Sprague–Dawley rats (400–450 g) were initially tested in the tail-flick procedure on each of two occasions: immediately before the first 30 min stress session (day 1), to obtain a baseline latency, and immediately following this stress session. Thereafter, these experimental animals were re-exposed to the 30 min stress session, once daily, for a total of 13 consecutive sessions. On day 13 the animals were again tested in the tail-flick procedure for pain responsiveness on each of two occasions in the manner previously outlined. Comparable numbers of non-stress controls were, likewise, tested for pain responsiveness at appropriate times on both days 1 and 13.

Stress was produced by a 30-min period of intermittent, inescapable foot shock. During each session, rats were individually placed on the grid of an operant chamber, where a 3-mA scrambled shock stimulus was delivered for 1-s duration, every 5 s.

The pain responsiveness test was a modification of the tail-flick measure of D'Amour and Smith[11], and is described elsewhere[12]. Briefly, it involves focusing radiant heat on the tip of the rat's tail, and measuring the latency of the withdrawal reflext. The radiant heat source was adjusted such that the majority of the animals responded between 3.5 and 4.0 s during baseline testing. A cut-off point of

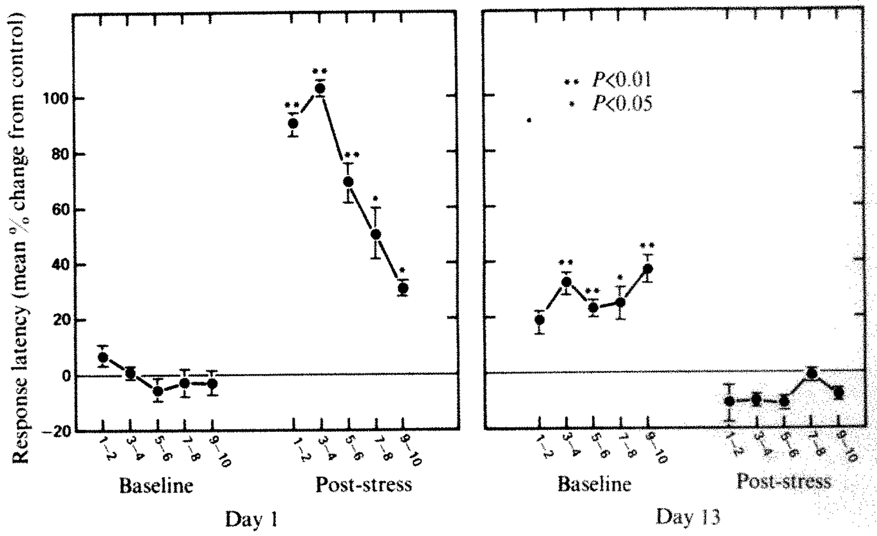

Fig. 1 Effect of acute and repeated stress on tail-flick latencies. Pain responsiveness was measured before (baseline) and after 30 min of stress (post-stress) on days 1 and 13. Response latencies of experimental animals ($n = 4$) were transformed into percentage change from control ($n = 5$) for each time point. Each point and corresponding bar represents mean ± s.e. for each pair of successive trials. Asterisks indicate significant differences from corresponding control trials.

6 s was imposed to prevent tissue damage. Ten trials were conducted during each test session, with 60–75-s intervals between trials.

To determine the possible effect of stress on endogenous opioid peptides, the levels of these peptides were measured in three separate experiments employing 33 animals. Since the results of these studies were internally consistent, they were subsequently pooled. The opioid levels were determined at four different time points. The initial two points were obtained by killing animals either before an acute stress session in order to measure control basal levels, or immediately following that stress session. The remaining two time points involved animals which had been subject to the repeated stress paradigm. These animals were killed on day 13 either before the stress session, in order to measure their resting levels, or immediately following stress. Endogenous opiate-like activity was measured using a ^{3}H-naloxone membrane binding assay[13,14]. A P_2 fraction was prepared from whole brain, and the peptides were then extracted by hypotonic lysis (10 mM Tris-HCl, *p*H 7.7, at 25 °C), boiled for 15 min, and centrifuged for 1 h at 100,000*g*. Extracts from 40 mg of tissue (wet weight were added to the binding preparation, and the inhibition of stereospecific opiate binding measured. Since we did not separate enkephalins from possible larger peptides, endogenous opiate activity was expressed in units, with one unit being defined as the amount which yields 50% receptor occupancy. All samples were assayed in duplicate; duplicate pairs differed by less than 10%. The endogenous opiate activity was abolished by treatment with carboxypeptidases, or by preincubation at 37 °C in the whole brain homogenates. Also, the effect of endogenous factors on stereospecific binding was enhanced by manganese and inhibited by sodium, indicating their specific and agonistic nature[13].

Acute stress (30 min) results in a significant increase ($P<0.001$) in tail-flick latency. As can be seen in Fig. 1 (day 1) analgesia is most dramatic during the first few trials immediately following foot-shock, with all experimental animals reaching the cut-off point (6-s response latency) on one or more of the initial trials. The pain responsiveness gradually recovers with successive trials. Nonetheless, these animals still remain significantly analgesic relative to non-stressed controls through the end of the testing.

Daily re-exposure to the 30-min stress session results in a significant elevation ($P<0.001$) in baseline response latencies when tested before the stress session on day 13. However, the subsequent stress session does not induce any further analgesia. On the contrary, it brings about a hyperresponsiveness to noxious inputs, such that post-stress latencies are significantly shorter than their pre-stress baseline responses, and significantly shorter than the response latency of non-stress controls.

Figure 2 displays the parallel changes in opioid peptides and pain responsiveness measured at various times throughout the stress paradigm. Opioid peptides are significantly elevated after a 30-min acute stress session (day 1) when animals exhibit profound analgesia. Daily re-exposure results in moderately elevated resting opioid levels and an intermediate degree of analgesia on day 13. However, following stress on day 13, these levels decrease, returning to control values. Similar effects are obtained with 60 min

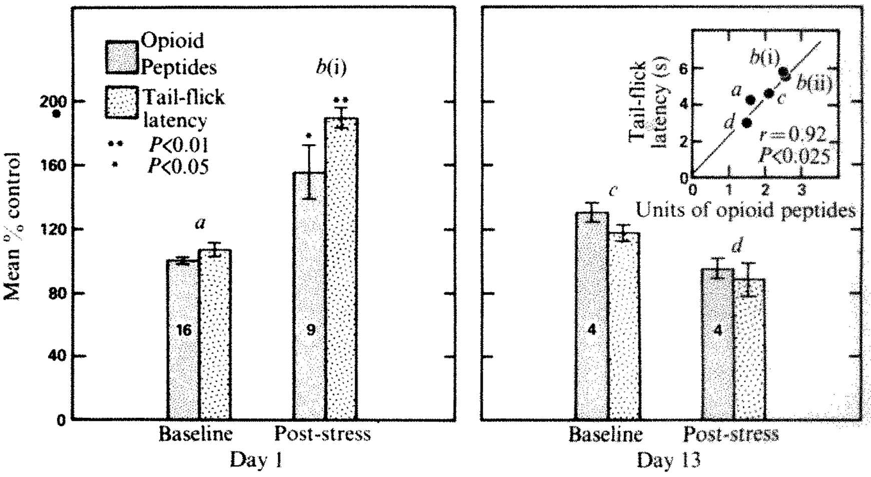

Fig. 2 Parallels between stress effects on pain responsiveness and opioid peptide levels. Opioid levels determined pre- and post-stress on days 1 and 13. Corresponding tail-flick latencies (trials 1 and 2 of each testing session, $n = 4$) for each time point are also displayed. Biochemical and behavioural data expressed as mean percentage control ± s.e. Number per group for opioid determination indicated at base of each bar. Inset demonstrates statistically significant correlation between units of brain opiate activity (per 40 mg wet weight) and tail-flick latencies (first two trials) under several stress conditions. *a*, Non-stress control. *b* (i), After 30 min acute stress; *b* (ii), after 60 min acute stress. *c*, Resting levels on day 13, after chronic 30 min stress. *d*, After 30 min repeated stress.

of acute stress, with experimental animals ($n=11$) exhibiting significant analgesia ($P<0.01$) and their brain opioid levels rising to 169% ($P<0.02$) of control levels.

The present results suggest a potential role of endogenous opiates in pain modulation and adaptive responses to stress. The levels of these peptides seem to rise with foot-shock stress, and this rise is accompanied by significant analgesia. This analgetic effect of acute stress has been independently described by other investigators[15]. While there is no *a priori* reason to attribute the full-blown analgesic effect of stress to the endogenous opiate system, it seems likely that this system may have an important role in the decrease in pain responsiveness observed after stress. Furthermore, repeated foot shock causes parallel changes in both opiate activity and behavioural responsiveness. Thus, the animals exhibit higher response latencies after chronic stress, and have slightly elevated resting levels of opiates. Yet further stress brings about a paradoxical decrease in response latencies, accompanied by a return of opiate levels to control values. It is apparent that the same stress has differential effects on behaviour and opioid levels, depending on the previous stress experience of the animal.

It should be noted that our biochemical measures involved overall opiate-like activity in the brain. While a large portion of this activity co-chromatographs with authentic enkephalins (Sephadex G-15, AG-1X2, 200–400, acetate form), it is quite possible that the larger brain opioid peptides recently described[3] may also be involved in the effects we report. Further, it would be of interest to assess the possible role of pituitary opioids—not measured here—in the stress response. While the specific interactions between brain and pituitary opiates have not been determined, their interplay may prove critical for stress adaptation and survival.

The mechanisms underlying the present findings remain to be determined; however, it seems reasonable that both physiological adaptive mechanisms and learning mechanisms may be involved in the phenomena we have observed. The biochemical events leading to increased opioid levels could involve increased synthesis, decreased breakdown, or activation of previously inactive forms. Furthermore, it is possible that different brain regions exhibit differential effects of stress. In any case, it seems likely that the rise in levels is indicative of a potentiation of the endogenous opioid system, since we have demonstrated in a separate experiment[10] that stress-induced analgesia is partially prevented by naloxone administration. This finding directly implicates opiate-receptor interactions in the change of pain responsiveness observed after stress. It therefore appears that the endogenous opiate peptides, by mediating some of the sensory changes that occur during stress, may provide an important interface between hormonal, sensory and emotional responses.

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Note added in proof: We have noted in subsequent experiments that, when enkephalins are separated from other opioid factors, specific enkephalin activity can exhibit changes which are different from those in overall opiate activity.

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Rapid eye movement cycle is a sleep-dependent rhythm

NORMAL nocturnal monophasic sleep is characterised by the cyclic alternation of rapid eye movement (REM) and non-rapid eye movement (NREM) sleep, with the REM phase of this cycle recurring approximately every 90–110 min. Kleitman[1] hypothesised that an analogous 90–110 min basic rest–activity cycle (BRAC) exists during the awake state, implying a biological clock that continues to operate with the same periodicity throughout the 24-h period (that is, an activity-independent clock). We report here on the effect of altered sleep–wake schedules on the REM cycle and present evidence that the REM cycle is sleep-dependent—it operates only when the organism is sleeping and is not an expression of an activity-independent rhythm.

Several studies[2–5] have supported Kleitman's BRAC hypothesis in identifying a 90–110-min cycle during waking in arousal level, perceptual illusions, gastric motility, and in some hormone secretions. It has been suggested[2–4] that these cycles found in the awake state are expressions of the REM cycle rhythm in sleep. Under this activity-independent model, any wake time occurring within the REM cycle would not alter the REM cycle period; the time between successive stage REM onsets would remain constant, regardless of the amount of sleep or waking within the cycle.

Under the sleep-dependent model, wake time occurring between stage REM onsets should lengthen the REM cycle period, since the clock would stop on awakening. Březinová[6,7], in studying the effects of spontaneous and externally imposed waking on sleep cycle length, reported that cycles interrupted by varying amounts of waking were significantly longer than intact cycles. Feinberg[8] compared the sleep of elderly subjects, which is characterised by frequent and sometimes prolonged awakenings, with that of younger subjects. He found the REM cycles in the two groups virtually identical when the intervening wake time was subtracted. In addition, the first REM onset in normal noctural sleep is invariably preceded by 60–120 min of non-REM sleep, despite varying sleep onset times. These findings imply that it is not simply total elapsed time that determines cycle length, but sleep time.

Altered sleep–wake schedules such as in napping, or multiphasic sleep, provide an opportunity to test the sleep-dependent hypothesis (Fig. 1). We have analysed the sleep of 25 adults, aged 17–40, from three different sleep laboratories who underwent altered sleep–wake schedules. Following one baseline night of normal sleep, 8 subjects (Naval Health Research Center, San Diego)[9], underwent a 60-min sleep–160-min wake schedule for 40 h. Ten subjects (Stanford University School of Medicine)[10], had two baseline sleep nights followed by a 30-min sleep–60-min wake

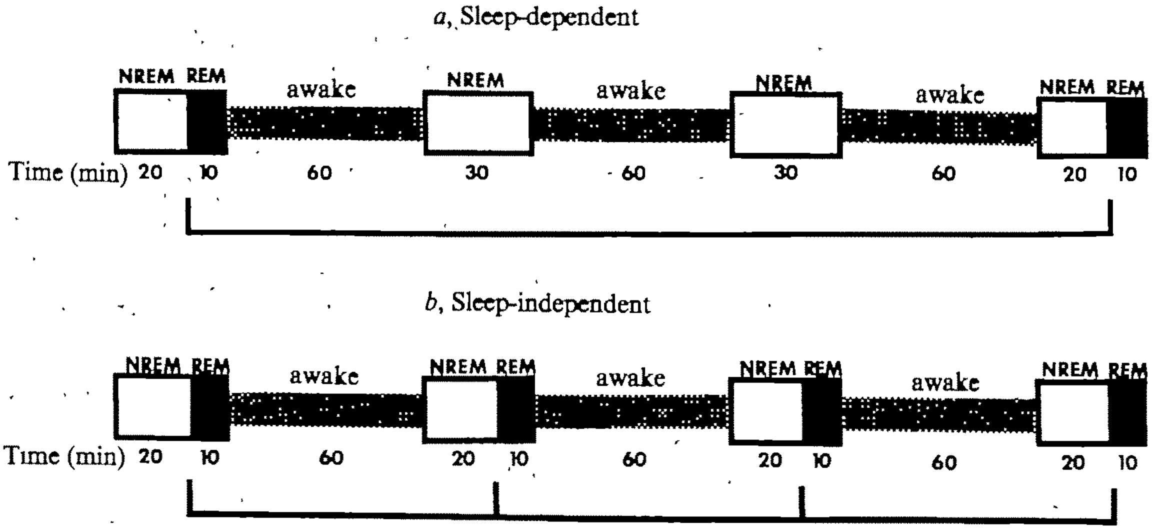

Fig. 1 Hypothetical examples of REM onsets occurring during a 30-min sleep—60-min wake schedule. *a*, Total sleep time between REM onsets is 90 min (total real time is 270 min). The 'clock' governing the time interval between REM onsets runs only during sleep; it stops on awakening and resumes at the next sleep onset. *b*, Total real time between REM onsets is 90 min (total sleep time is 30 min). The 'clock' runs during both sleep and waking. Studies of nap sleep from three independent sleep laboratories support the sleep-dependent model[1]

schedule for 5 d. Seven subjects (Montefiore Medical Center)[11], slept normally for 7 baseline nights and then were placed on a 60 min sleep–120 min wake cycle for 10 d.

Table 1 REM cycle periods for nap and baseline sleep conditions

	San Diego $N=8$		Stanford $N=10$		Montefiore $N=7$	
Nap RCP	96.6	(±20)	79.1	(±23)	119.2	(±30)
Baseline RCP	97.1	(±12)	92.0	(±12)	104.6	(±12)
Nap-Baseline RCP	−0.50	(±21)	−12.9	(±24)	14.6	(±31)
	Not significant		Not significant		Not significant	

If the REM cycle clock is sleep-dependent, stage REM onset should appear only after 90–110 min of sleep has accrued since the last REM onset, regardless of the amount of wake time between or within the scheduled sleep periods. Using the last REM onset on the last baseline night as a starting point, we 'compressed' the sleep during naps for each group of subjects by subtracting all intervening wake time, and viewed this sleep as one continuous sleep period. For both baseline and naps, a REM cycle period (RCP) was defined as the sleep time between two successive stage REM onsets

According to the sleep-dependent hypothesis, the RCP in naps should not differ significantly from the baseline night RCP. We computed the average difference between nap and baseline RCP for each subject, and the overall average difference was tested for significant (0.05 level) deviation from zero with a *t* test. None of the mean differences differed significantly from zero (Table 1).

Was this sleep-dependent rhythm only present during a specific phase of the circadian sleep–wake cycle? To answer this question, we combined the 3 studies and compared nap REM cycles starting during the time period in which sleep usually occurs (2000–0800) with those cycles starting during the normal wake period (0800–2000). An equal number of naps occurred in the two 12-h periods. The REM cycle durations for the 0800–2000 and 2000–0800 time periods were 87.3 min and 88.6 min respectively, a non-significant difference.

These findings suggest that (1) the 90–110-min cycle found in some waking activities is not an expression of the REM cycle found in sleep, but is a separate neurophysiological process, (2) the nap REM cycle period is not modulated by the circadian sleep–wake cycle, and (3) the REM cycle clock is sleep-dependent.

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Endorphin from pituitary inhibits cyclic AMP formation in homogenates of neuroblastoma × glioma hybrid cells

OPIATES inhibit basal and prostaglandin E_1-stimulated formation of cyclic AMP by homogenates of cultured neuroblastoma × glioma hybrid cells[1,2]. The opiate inhibition is stereospecific and is blocked by the specific opiate antagonist naloxone. Thus, interaction of an opiate agonist with its receptor inhibits adenylate cyclase activity.

Peptides with opioid activity (endorphins) have been found in the pituitary of several species[3,4] and have been partially purified[5]. The pituitary peptide β-lipotropin (β-LPH) gives rise to biologically active fragments containing a pentapeptide sequence (methionine–enkephalin) with opioid activity at the NH_2-terminus[6], and this pentapeptide has also been found in brain[7]. In porcine pituitary, the principal opioid activity is associated with peptides that differ from all the active β-LPH fragments in that their biological activity is degraded by trypsin, as measured in the guinea pig myenteric plexus–longitudinal muscle bioassay[8]. Further characterisation of these endorphins is presented elsewhere[9]. Since endorphins derived from pituitary interact with opiate receptors in binding assays and have typical morphine-like actions in bioassays[5,10,11] it was of interest to determine if this pituitary endorphin had typical opioid effects upon adenylate cyclase activity.

The source of the endorphin was the 'MSH concentrate' from porcine pituitary described elsewhere[5]. The guinea pig myenteric plexus-longitudinal muscle[12] was used to assay all fractions during purification. A 'guinea pig unit' (GU) is defined as the amount of material giving naloxone-reversible inhibition equivalent to that produced by 1 nmol of normorphine in the 5-ml tissue bath (that is, 200 nM final concentration). Half-inhibition of the muscle twitch amplitude requires approximately 0.5 nmol normorphine (100 nM). The crude material was first fractionated on a BioRex-70 cation exchanger (BioRad Labs., Richmond, Ca, column size 4×90 cm); activity was obtained in a basic peak eluting with 0.25 M ammonium carbonate. The active fraction was passed through a BioGel P-6 column (2.5×52 cm) in Tris-HCl buffer (50 mM) at *p*H 7.4; activity was found in a peak with apparent molecular weight about 3,000. This material was refractionated on BioGel P-6 in distilled water, in order to take advantage of adsorptive properties of the column for peptides at low ionic strength. The activity peak emerging from this column had specific activity 132 GU/A_{274} Unit, whereas the specific activity of the starting material was 7.2 GU/A_{274}. (A_{274} Unit=amount of material in 1 ml solution with 1 cm light path giving absorbance 1.0 at 274 nm, the tyrosine extinction maximum.) The endorphin was concentrated on an Amicon UM05 filter (Amicon Corp., Lexington, Mass.) and portions were lyophilised and shipped in sealed tubes from Palo Alto to Bethesda.

This endorphin preparation was still impure, as evidenced by the presence of several ninhydrin spots on thin-layer chromatography plates developed in n-butanol : acetic acid : water (1 : 1 : 1, v/v/v). However, it is of interest because, as documented elsewhere[9], it represents most of the opioid activity in the pituitary, it exhibits high potency, and it is clearly different from any active fragment of β-lipotropin.

The procedure used to measure adenylate cyclase activity in neuroblastoma × glioma NG108-15 hybrid cell homogenates was as described by Sharma *et al.*[1].

Table 1 shows the effects of this pituitary endorphin on adenylate cyclase activity of the neuroblastoma × glioma NG108-15 hybrid cell homogenates. Endorphin was a more potent inhibitor of basal and PGE_1 stimulated adenylate cyclase activity than was morphine. Here the potency of the pituitary endorphin was 10 times that of morphine when the peptide concentration was calculated from absorbance at 274.5 nm, assuming one tyrosine per mole. Based upon normorphine equivalents in the guinea pig myenteric plexus bioassay, the potency inhibiting adenylate cyclase was 100 times that of morphine.

An experiment similar to that shown in Table 1 was performed also with a different endorphin preparation, containing material of about 1,750 daltons obtained from a commercial porcine crude ACTH preparation[4]. The results were virtually identical to those reported here for the 3000-dalton endorphin from MSH concentrate.

Fig. 1 shows the concentration-dependent blockade by naloxone of the inhibition of adenylate cyclase by endorphin in neuroblastoma × glioma cell homogenates. Complete antagonism of the endorphin effect could be achieved, except at the highest endorphin concentrations, and the curves are generally indicative of the expected competitive agonist-antagonist relationship. Using the method of Kosterlitz[13], K_e for naloxone can be estimated as approximately 5×10^{-8} M, in reasonably good agreement with that obtained in this system with morphine (2×10^{-8} M) or methionine-enkephalin (3×10^{-8} M) (W. Klee and M. Nirenberg, unpublished observations).

Table 1 Effect of pituitary endorphin on adenylate cyclase of neuroblastoma × glioma cell homogenates

	Addition	Cyclic AMP formed (pmol min^{-1}mg^{-1} protein) —Naloxone	+Naloxone (10μM)	% of control —Naloxone	+Naloxone (10 μM)
Basal	None	25	24	100	97
	Morphine (10 μM)	14	23	56	95
	Endorphin (0.016 μM)	18	24	72	96
	Endorphin (0.16 μM)	13	23	52	92
	Endorphin (1.6 μM)	11	18	44	72
+ PGE_1 (0.25 μM)	None	265	275	100	104
	Morphine (10 μM)	192	262	72	99
	Endorphin (0.016 μM)	263	269	99	102
	Endorphin (0.16 μM)	212	272	80	103
	Endorphin (1.6 μM)	174	256	66	97

Procedure of Sharma *et al.*[1]. Incubations were for 5 min at 37 °C with the following components in a final volume of 100 μl: 15 mM Tris-HCl (*p*H 7.5); 1 mM α-^{32}P-ATP, 10^6 c.p.m.; 1 mM ^{3}H-cyclic AMP, 10^4 c.p.m.; 5 mM $MgCl_2$; 20 mM creatine phosphate; 0.5 mM RO20–1724 as cyclic AMP phosphodiesterase inhibitor (0.1% ethanol final concentration) creatine phosphokinase, 160 units mg^{-1}, 10 units; 0.16 M sucrose; 0.097 mg NG108–15 homogenate protein (frozen cells stored at –80 °C in 0.32 M sucrose, 0.01 M Tris-HCl, *p*H 7.5). Endorphin concentrations are normorphine equivalents, according to activity assayed in the guinea pig myenteric plexus-longitudinal muscle preparation, where normorphine and morphine have equivalent potencies. Data are means of duplicates from a representative experiment that was repeated three times.

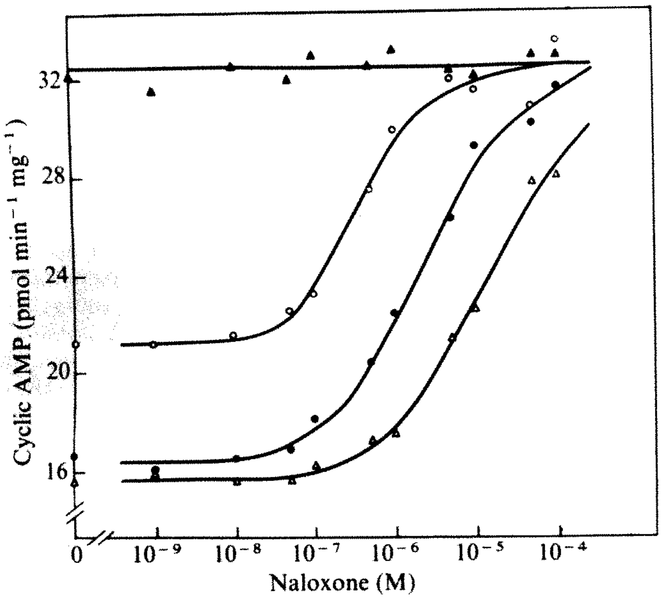

Fig. 1 Naloxone blockade of the endorphin inhibition of adenylate cyclase in neuroblastoma × glioma cell homogenates. Method as in Table 1. ▲, Control, no endorphin; ○, 0.033 μM endorphin (as normorphine equivalent in myenteric plexus-longitudinal muscle bioassay); ●, 0.33 μM endorphin; △, 3.3 μM endorphin.

Our results indicate that pituitary endorphin has typical opioid effect on adenylate cyclase of neuroblastoma × glioma NG108-15 homogenates. Since opioid peptides of molecular weight in the 3,000-dalton range, possibly identical to pituitary endorphins, are found in brain[14], it is likely that our findings have physiological significance for the function of endorphins in the nervous system.

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Feedback loop or output pathway in striato-nigral fibres?

THE turnover of dopamine has been thought to be controlled by a 'feedback' pathway from the postsynaptic dopamine receptors in the striatum to the dopamine-containing cells in the substantia nigra[1]. This concept formed the basis of an explanation of the effects of neuroleptics on dopamine turnover[1-3]. Evidence for a striato-nigral α-aminobutyrate (GABA)-containing pathway[4,6] has supported the idea although there have been dissenting voices[7,9]. In particular, the discovery of dopamine receptors on the dopamine-containing cells themselves[8,10] has led to the suggestion that these nigral receptors might explain results otherwise explained by the feedback hypothesis[9]. Nevertheless, the existence of these receptors does not exclude the action of feedback loop in the control of dopamine turnover. We report here an attempt to evaluate the action of the striato-nigral pathway in the control of dopamine metabolism. Our results suggest that lesions in the GABA-containing striato-nigral pathway, which spare the dopamine-containing fibres of the nigro-striatal system, do not affect dopamine metabolism in the nigro-striatal dopamine neurones or the increase in dopamine turnover after administration of haloperidol.

Radioactive L-leucine, injected into the brain, is taken up rapidly by neuronal cell bodies in the region of the injection site, incorporated into proteins and transported along the axons of the cells[11]. Output fibres from a given set of neurones can thus be traced autoradiographically[12] and after injections of 4,5,^{3}H-L-leucine into the straitum the route followed by the striato-nigral fibres was traced in the brains of six albino Wistar rats. At the level of the hypothalamus shown in Fig. 1, the radioactivity marking the axons of this pathway is wholly contained within the internal capsule, well separated from the dopamine-containing fibres of the nigro-striatal system which have not entered the capsule at this level[13].

An initial series of animals with 6-mC electrolytic lesions aimed at this area could be divided into two groups (Fig. 2). One group characterised by small well localised lesions showed a marked turning response towards the lesioned side with both apomorphine (0.5–5 mg kg^{-1}) and amphetamine (2 mg kg^{-1}) in spite of intact dopamine neurones assessed both biochemically and in the fluorescence microscope. The other group of rats showed no turning response and their lesions were located outside the internal capsule.

A further 12 rats showing the turning response to apomorphine after electrolytic lesions in the striato-nigral pathway were used to assess the effect of the lesion on the biochemical parameters of dopamine turnover. Using modifications (unpublished results of N.M.N.) of the published GLC methods[14] we have measured the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) in striatum and of GABA in the substantia nigra. The striatal dopamine concentration was also measured by a sensitive radiochemical enzymatic assay[15]. Previous experiments have shown that GABA levels in nigra drop by 50% after hemisections of brain between striatum and nigra[4]. Table 1 shows that although the lesions were as effective as complete hemisections in reducing the concentration of GABA in nigra, the response of the dopamine system to haloperidol is not substantially different on the lesioned side from that on the intact side. Indeed there are are no significant differences between the lesioned animals and normal animals in any of the measures of dopamine metabolism.

Published evidence[16] and our own studies seem to indicate only two outputs from the striatal cells as seen by autoradiography. One goes direct to substantia nigra, and the

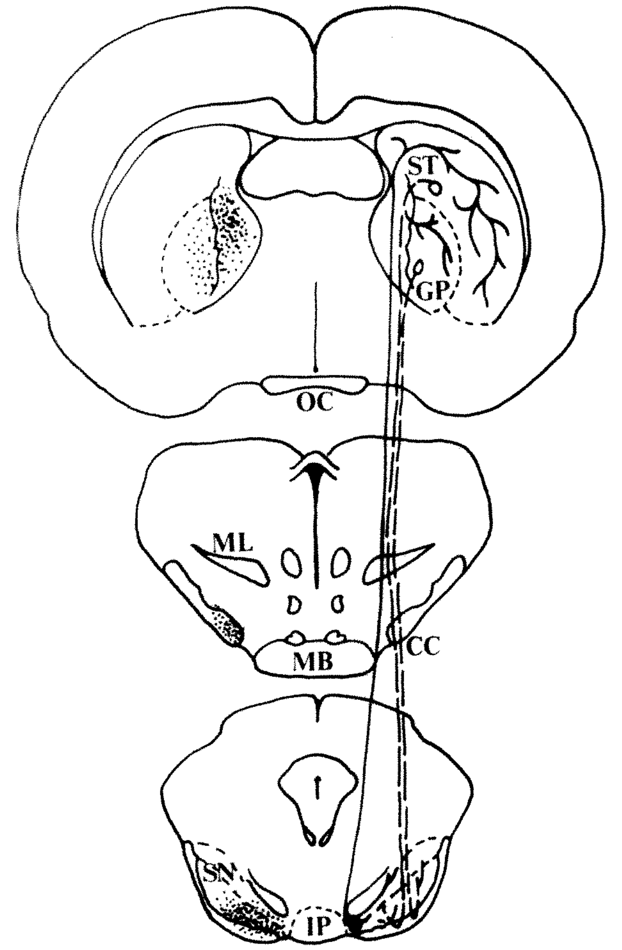

Fig. 1 The striato-nigral (GABA) and nigro-striatal (dopamine) system in rat brain. The dotted areas on the left of the sections summarise the distribution of autoradiographically localised silver grains 4 d after the injection of ^{3}H-leucine into the striatum. On the right are represented the pathways to and from striatum and substantia nigra reported in the literature[13-17]. The solid lines and filled cell body represent the nigro-striatal dopamine system; the open circles and dotted lines represent the GABA containing striato-nigral systems. At the level of the mamillary bodies represented by the middle section the GABA fibres run separately from the DA ones which enter the internal capsule at a level anterior to this. GP, Globus pallidus; CC, crus cerebri; IP, interpenduncular nucleus; MB, mamillary bodies; ML, medial lemniscus; OC, optic chiasma; SN, substantia nigra, and ST, striatum.

other synapses in the globus pallidus. There are GABA-containing fibres projecting from pallidum to nigra[16] but the pathway which they follow is very close to the site of our lesion and this, in conjunction with the fall in GABA levels, suggests that we have extensively damaged the documented pathways from striatum to nigra, with no effect on the control of striatal dopamine metabolism in either normal animals or in animals treated with haloperidol.

It is conceivable that any remaining neurones of the strial-nigral or of the pallido-nigral pathways could have functionally compensated for the reduction of GABA content in the substantia nigra by an increase in their responsiveness to neuroleptics. In addition to such a presynaptic adaptation, the GABA receptors in substantia nigra could develop denervation supersensitivity and compensate for the damage resulting from the lesions. Further work is required to demonstrate these possible compensatory mechanisms in the striato-nigral systems. Our present interpretation is that the striato-nigral pathway is not necessary for control of striatal dopamine turnover.

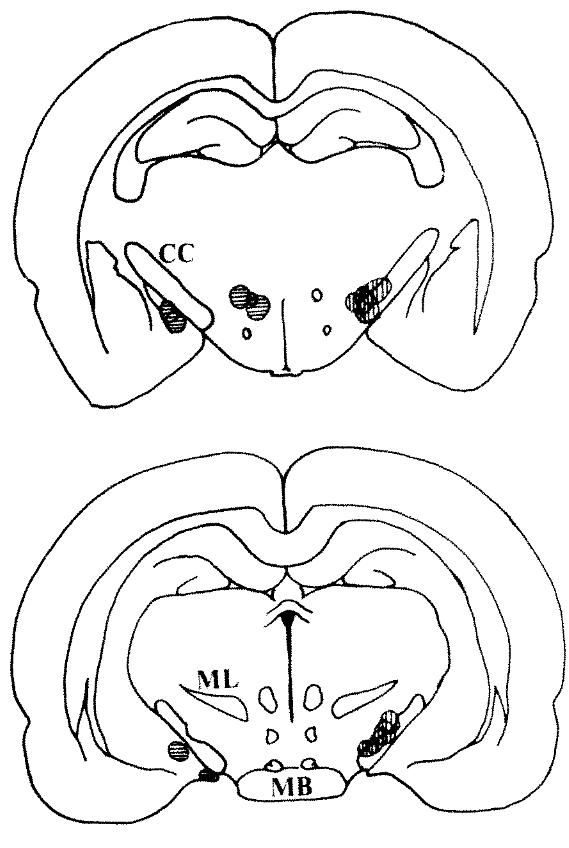

Fig. 2 Lesion sites from the first 14 rats projected on the coronal sections of the rat brain redrawn from ref. 22. The drawings represent sections 1 mm apart. The vertically-hatched lesion sites on the right are from eight rats which turned after apomorphine treatment (2 mg kg i.p.). The horizontally hatched areas on the left represent the maximal extent of lesion sites in six animals which failed to show turning behaviour in response to the same dose of apomorphine. The abbreviations are those used in Fig. 1.

Table 1 Effect of striato-nigral pathway lesions on striatal dopamine metabolism and GABA concentrations in substantia nigra in untreated and haloperidol-treated rats

		GABA substantia nigra	Concentration in stratum Dopamine	HVA	DOPAC
Untreated lesioned animals	Lesioned side	117±23 (4)*	9.4±0.49 (8)	1.2±0.11 (8)	1.1±0.06 (8)
	Intact side	236±54 (4)	9.2±0.58 (8)	1.4±0.13 (8)	1.2±0.11 (8)
Lesioned animals 30 min after 1 mg per kg haloperidol	Lesioned side	147±20 (8)*	5.2±0.46 (8)†	4.6±0.41 (8)†	3.3±0.21 (8)†
	Intact side	319±58 (8)	6.1±0.53 (8)†	4.9±0.51 (8)†	3.1±0.2 (8)†

Concentrations are given as μg per g wet weight ±s.e. (n). 2–3 Weeks elapsed between lesion induction and the death of the animal. An area of brain including substantia nigra (SN) was dissected free hand on ice and frozen in liquid nitrogen 2–4 min after the death of the animal, when the post-mortem increase in GABA should have been complete[23]. GABA concentrations in the table are uncorrected for recovery which was approximately 60%. Striata from the same brains were dissected on ice after the nigral dissection and frozen 4–8 min after decapitation. Estimates of HVA and DOPAC concentrations are also uncorrected for recovery but it was approximately 95%. Dopamine concentrations are corrected.

*GABA concentrations are significantly lower on the lesioned side, $P < 0.02$ (paired t test, two-tailed).

†These concentrations are all significantly different from the appropriate control values (in untreated animals), $P < 0.001$ (t test, two-tailed).

Speculation as to the function of the striato-nigral pathway is perhaps in order if it does not subserve the 'feedback' role usually designated to it. After induction of unilateral lesions in the nigro-striatal dopamine neurones with 6-hydroxydopamine, apomorphine in very low doses causes circling behaviour away from the lesioned side[17]. This behaviour is abolished by ipsilateral electrolytic lesions[18] located in a similar site to that shown in Fig. 2. Animals with both nigro-striatal and the striato-nigral systems lesioned unilaterally show a turning behaviour similar to that reported after the striato-nigral lesions alone[18]. The turning behaviour seen after extensive unilateral ablations of the striatum is also similar in direction and drug sensitivity[19]. That is, the induction of unilateral lesions of the striato-nigral pathway, with or without damage to the dopamine neurones is equivalent to striatal ablation in its effect on turning behaviour. We have recently found deficits in passive avoidance behaviour in animals with bilateral lesions in the striato-nigral system, which is also similar to the deficits seen after large bilateral lesions[20].

Since the striato-nigral pathway terminates predominantly within the zone reticula region of substantia nigra it seems likely that it influences neural activity within this region. The recent electrophysiological evidence for a functional connection between striatum and thalamus through zona reticulata neurones[21] suggests that the fibres which we have lesioned are a part of this system rather than a feedback loop controlling dopamine neurones in zona compacta of substantia nigra.

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Can chloropractolol alkylate β adrenoceptors?

EREZ *et al.*[1] have classified chloropractolol as an irreversible, covalently-binding, antagonist of cardiac β adrenoceptors. This conclusion was based on the knowledge that similar halogen amides can alkylate sulphydryl groups[2,3] and their observation that chloropractolol produced a long-lasting antagonism of β adrenoceptors associated with a depression of the maximum response to isoprenaline.

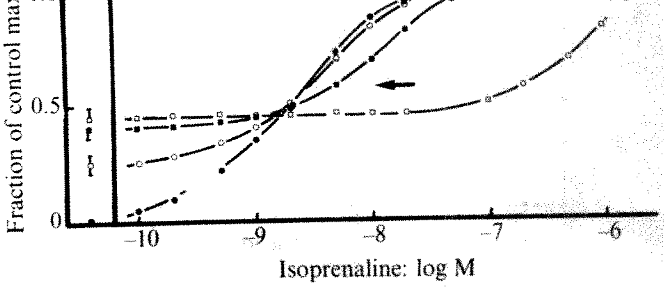
We have measured the contraction frequency of isolated rat atria, the system used by Erez *et al.*, and have found that (±) chloropractolol has agonist activity of its own with half-maximal response at 4×10^{-8} M ($n=7$) which is about five times the concentration for half-maximal response to (±) isoprenaline on this tissue. The increase in atrial frequency due to chloropractolol was not reduced by pretreatment with reserpine but was promptly antagonised by (±) propanolol, 2×10^{-8} M. The maximum agonist response to chloropractolol, however, was 0.41 ± 0.02 ($n=8$) times the isoprenaline maximum (see control value in Fig. 1 for 10^{-5} M chloropractolol alone). This compares with a maximal response for (±) practolol of 0.26 ± 0.04 ($n=4$) times the isoprenaline maximum.

We have found that chloropractolol also has antagonist properties and therefore can be classified, like practolol[4], as a partial agonist. Dextrad displacement of the isoprenaline dose-response curve, produced after equilibration for 45 min with 10^{-5} M chloropractolol, is shown in Fig. 1.

The shift of the isoprenaline dose–response curves, or dose-ratio (DR), has to be estimated for a partial agonist from the difference in the logarithms of the concentration of isoprenaline producing half-maximal responses as described by van Rossum[5]. Dose ratios for isoprenaline were obtained after equilibration with chloropractolol concentrations of 10^{-8} to 10^{-5} M. A Schild plot of log (DR-1) against log [B], the chloropractolol concentration, gave a linear regression with a slope of 0.94 (95% confidence limits 0.88–1.0), which is not significantly different from unity. The estimated dissociation constant for chloropractolol was 1.2×10^{-8} M (95% confidence limits of 8.9×10^{-9} to 1.5×10^{-8} M). No difference in the apparent dissociation constant for chloropractolol was found when comparing atria from untreated rats or rats pretreated with reserpine. Our estimate of the apparent dissociation constant for practolol, based on similar experiments, was 9.8×10^{-8} M (95% confidence limits of 2.7×10^{-8} to 1.9×10^{-7} M) derived from a Schild plot of slope 1.2. The slope for practolol was not significantly different from that for chloropractolol.

In Fig. 1, the control log dose–response curve to isoprenaline has been normalised by expressing measured increases in atrial frequency as fractions of the maximal increase in frequency. The isoprenaline log dose–response curve

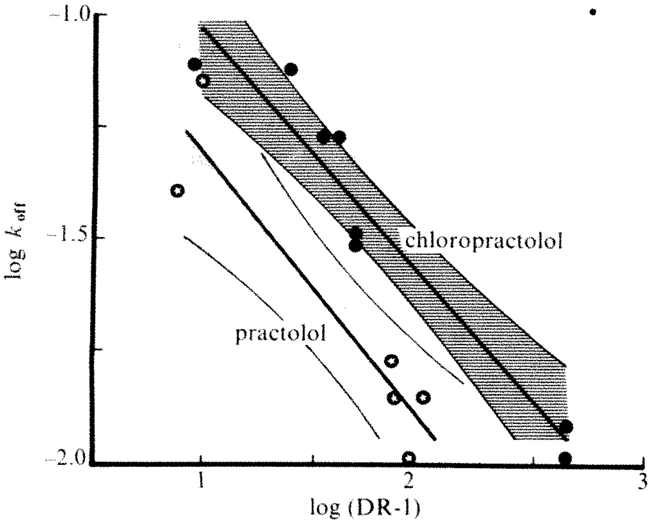
Fig. 1 Dose–response curves to isoprenaline in isolated rat atria. Ordinates: increase in atrial rate as a fraction of the maximal response to isoprenaline. Abscissae: log molar concentration of isoprenaline. The results are the average of four experiments. The curves shown are: isoprenaline alone (●); repeated after equilibration with 10^{-5} M chloropractolol for 45 min (□); repeated after four changes of bath fluid (30 ml) during 35 min (■); and repeated after another six changes of bath fluid during 90 min (○). Basal rates to chloropractolol alone are shown separately.



obtained in the presence of the antagonist was found by expressing measured increases in atrial frequency produced by the combined agonist effects of chloropractolol plus isoprenaline as fractions of the control maximum increase in frequency due to isoprenaline alone. If, instead, the increases in frequency produced by chloropractolol are ignored and the results are presented as described by Erez *et al.* (where increases in atrial frequency due to isoprenaline in the presence of antagonist are expressed as fractions of the control isoprenaline maximal increase), then a family of curves is generated with progressively reduced maxima giving a spurious appearance of non-competitive antagonism. Erez *et al.* make no reference to agonist activity of chloropractolol and their dose–response curves may have been plotted using data normalised in a misleading way. Until this problem of the agonist effect of chloropractolol altering isoprenaline dose–response curves is solved, it might be prudent to put more weight on the time-course of effect as an indicator of 'irreversibility'.

An indication of the time course of recovery from blockade can be obtained from Fig. 1. The time course of the offset of antagonism can be followed by estimating $p = \mathrm{DR}-1/\mathrm{DR}$ which is a function of fractional receptor occupancy, as defined by Paton[6] and Schild[7]. In our experiments, dose ratios were measured from complete dose–response curves, so no more than three estimates could be made in each washout sequence. Nevertheless, plots of log p against time were close to linearity in each experiment, suggesting that first-order kinetics are an adequate description of the reversal of blockade. Rate constants for recovery from blockade, k_{off}, were calculated from these plots and their relation to initial dose ratio in each experiment is shown in Fig. 2. The slopes of the log $(\mathrm{DR}-1)$ against log k_{off} regression lines for both chloropractolol and practolol are significantly different from zero; therefore k_{off}, which is apparently dependent on initial receptor occupancy, cannot be taken as an estimate of the dissociation rate constant for the drug–receptor complex. If the tissue contained other high-affinity binding sites for these antagonists so that, during washout, the receptors could be supplied with drug by slow efflux from this other pool then a similar dependence of k_{off} on concentration would be found. Although the dissociation of the drug–receptor complex is unlikely to be the rate-limiting step in our system, estimated k_{off} is useful for comparing chloropractolol with practolol. Figure 2 shows that for equiactive concentrations of the antagonists, the k_{off} is significantly greater for chloropractolol than practolol and therefore there is no support here for an alkylation hypothesis. Depending on the initial concentration of antagonist the blockade washes out at a rate between 1% and 10% per min; no conclusion about alkylation can be based on this relatively slow washout because propanolol (10^{-8} M) washes out at a similar rate of 1–2% per min from isolated guinea pig atria[8].

Fig. 2 Time course of washout of antagonism of isoprenaline (for both chloropractolol and practolol) as a function of the initial degree of antagonism. This relationship has been linearised by expressing (on the abscissa) initial antagonism as log $(\mathrm{DR}-1)$, as used in the Schild equation (where DR = dose ratio), and by expressing, on the ordinate, the rate constants for washout as log k_{off}. Each rate constant was calculated from the apparent first-order kinetics shown by the linear regression of log p on time; p was calculated from the function $\mathrm{DR}-1/\mathrm{DR}$. Analysis of variance showed that the slopes of both lines are significantly different from zero but not significantly different from each other ($t = 0.39$). Analysis of covariance showed that the data are best described by separate parallel straight lines; for vertical difference between values of Y at given x, $t = 5.52$.



Thus, we have found that chloropractolol is a partial agonist at β adrenoceptors with about 1.5 times the intrinsic activity and 10 times the affinity of practolol. We can find, however, no evidence that chloropractolol alkylates β adrenoceptors in rat atrial muscle, and it is unlikely to be any more useful than other β adrenoceptor antagonists as a tool in receptor isolation studies.

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Histamine blocking agent in the salivary gland homogenate of the tick *Rhipicephalus sanguineus sanguineus*

STUDIES of reactivity to tick bites and tick salivary gland homogenate have revealed responses similar to those induced by histamine in the skin of lop-eared rabbits[1]. Salivary gland homogenates of the tick *Rhipicephalus sanguineus sanguineus*, however, did not cause contraction of guinea pig ileum, but blocked the action of histamine acid phosphate (HAP). We have therefore tested this extract against other drugs known to cause contraction of guinea pig ileum in order to discover the spectrum of its blocking effect on this tissue. We have found that the salivary gland homogenate of this tick antagonises and potentiates, respectively, the action of histamine and acetylcholine on guinea pig ileum.

Adult ticks at various stages of engorgement but never fully engorged were collected from dogs at a veterinary clinic and used within 18 h. They were washed in 1% merthiolate solution, dried on filter paper and stuck to the surface of low-melting-point (30 °C) wax by pressing the ventral surface down into the partially melted surface in a Petri dish and submerging the mouthparts and legs. The dish with the ticks was then placed in a deep freezer (−18 °C) for at least 1 h to harden the wax. The ticks were dissected in cold physiological saline (4 °C), and the

Table 1 Results of pharmacological tests on the salivary gland extract (SGE)

Substance	Concentration	Height of contraction of guinea pig ileum before administration of of extract (mm)	Height of contraction of guinea pig ileum after administration of 0.4 ml SGE (mm)	Reduction in contraction of guinea pig ileum (mm)	% Increase or decrease (Time)
Histamine*	$0.1\ \mu g\ ml^{-1}$	27.5	6.8	20.8	75.5
Histamine	$1.0\ \mu g\ ml^{-1}$	37.0	23.0	14.0	37.84
Barium chloride	40 mM	44.0	35.0	9.0	20.51
Acetylocholine	$0.1\ \mu g\ ml^{-1}$	25.0	31.5	6.5	26.0†

*Mean of data from two animals' tissues.

†There was an increase in contraction of the ileum following the application of acetylcholine ($0.1\ \mu g\ ml^{-1}$) after the addition of the salivary gland extract (SGE).

glands were extracted and put into a thick sterilised watch glass containing cold physiological saline. The salivary glands were freed (as much as possible) of bits of tracheae, salivary ducts and other tissues. After washing in two changes of cold physiological saline, the glands were homogenised in the watch glass with cold physiological saline by means of a sterilised glass rod with a ground end. To each pool of 25 pairs of salivary glands 0.4 ml of cold physiological saline was added. The homogenate was then removed into a sterilised Bijou bottle which was swirled gently to mix the contents. The dissection of the ticks and handling of the glands and homogenate was as far as possible carried out in sterile conditions. The homogenate was used as soon as possible.

Pharmacological testing of the salivary gland homogenate was carried out as described by Perry[5], on guinea pig ileum preparation in an organ bath containing Krebs solution at 37 °C aerated with a mixture of 95% oxygen and 5% carbon dioxide. Isotonic contractions were recorded on a physiograph (Narco Bio-Systems, Houston) using a microdisplacement transducer. Responses of the ileum to histamine acid phosphate ($0.1\ \mu g\ ml^{-1}$, $1.0\ \mu g\ ml^{-1}$), acetylcholine ($0.1\ \mu g\ ml^{-1}$) and 40 mM barium chloride were tested. The effects of the homogenate alone and against the action of histamine, acetylcholine and barium chloride on guinea pig ileum were also tested.

As before[2], 0.4 ml of salivary gland homogenate itself did not contract guinea pig ileum. But it antagonised the contraction of guinea pig ileum preparation caused by HAP (0.1 and $1.0\ \mu g\ ml^{-1}$) by 75.5 and 37.84%, respectively. Similarly, 0.2 ml of the homogenate reduced the contraction caused by HAP ($0.1\ \mu g\ ml^{-1}$) by 41.8%. The first test was reproducible (Table 1). The blocking action of the homogenate persisted for some time because the ileum did not contract after the homogenate had been washed off twice with Krebs solution and more HAP ($0.1\ \mu g\ ml^{-1}$) had been added. This indicates that the extract binds firmly to the tissues of the guinea pig ileum. After 2 h of washing, however, HAP ($1.0\ \mu g\ ml^{-1}$) produced a contraction of 35 mm. After removal of this and addition of HAP ($0.1\ \mu g\ ml^{-1}$) there was a contraction of 30 mm which was comparable to the original contraction of 27.5 mm due to the same concentration of HAP.

We showed that the agent in the tick's salivary gland homogenate was not an effective general blocking agent by its inability to antagonise substantially the contraction of the guinea pig ileum caused by addition of 40 mM barium chloride. On the contrary, the homogenate potentiated by 26% the contraction caused by acetylcholine ($0.1\ \mu g\ ml^{-1}$).

The pharmacological test showed conclusively that the salivary gland of *R. s. sanguineus* contains a histamine blocking agent. This emphasises the conclusion that the pharmacologically active substance detected in the saliva of this tick and that of *Haemaphysalis spinigera*[2] is either histamine or closely related to histamine.

The advantage to ixodid ticks in having both a histamine-blocking agent and histamine or histamine-like substance in their saliva is clear. Ixodid ticks attach to their hosts for a relatively long time and so in the absence of a histamine-blocking agent in the ticks' saliva far more pharmacologically active substance would be released into the host's tissues than necessary.

Excessive amounts of histamine in the host's tissues will endanger the life of the host to the ultimate disadvantage of the tick. On the other hand, the presence of a histamine-blocking agent in the tick's saliva implies an efficient mechanism for regulating the amount of histamine in the host's tissues at any particular moment to the advantage of the tick; perhaps small amounts being released during the first period of slow engorgement and larger amounts being released during the final period of rapid engorgement when such amounts may be required to cause a more intense reaction in the host, leading to the extravasation of a large quantity of blood.

The potentiating effects of the salivary gland extract on the action of acetylcholine on guinea pig ileum suggests that this extract would potentiate the effect of acetylcholine at the neuromuscular junction. If this effect becomes excessive at the motor end plate, it would lead to motor paralysis, which has been observed both in humans and animals after attachment and engorgement of some species of ixodid ticks[1,3]. Motor paralysis after the attachment of *Dermacentor andersoni* has been shown to be due to blockade at the neuromuscular junction[4,6].

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Mutual repression of synaptic efficacy by pairs of foreign nerves innervating frog skeletal muscle

CONSIDERABLE attention has been directed recently at the proposed phenomenon of synaptic repression. In lower vertebrates experimentally crossed nerves will successfully innervate inappropriate muscles; in certain cases, it has been observed that some fibres later regrow to their original muscles, where they form functional synapses. In some instances where this occurs, the foreign innervation then seems to lose effectiveness in making the muscle contract[1-3]. Mark and his collaborators have concluded that repressed synapses of the foreign nerve remain morphologically intact[4-6] and are capable of being reactivated rapidly if the original nerve is again cut[7-9]. Although other workers[10,11] have shown persistent double innervation without apparent repression in the same or comparable preparations in fish, Yip and Dennis[12] have recently confirmed the existence of quickly reversible synaptic repression in salamander limbs. There have been suggestions that similar synaptic repression might exist in the mammalian central nervous system[13]. If this is confirmed, it could constitute an important mechanism for plasticity during development or experience[14,15]. Thus it is important to determine the mechanism of repression and to assess the reasons for the variability in the degree of competitive interaction observed in different preparations.

Yip and Dennis[12], supporting the observations of Mark[9], report a sharp drop in the quantal content of foreign nerve EPPs in salamander muscle, but no change in miniature EPP (mEPP) size. In that preparation, as in the fish muscles used, both foreign and original nerves form distributed endings along much of the length of the muscle fibres, making it difficult to identify and study the properties of individual nerve endings. In the experiments described below we find evidence of mutual repression of synaptic effectiveness by two foreign nerves innervating frog skeletal muscle, where synapses are located far apart on muscle fibres and individual synapses can be examined. In these cases the competitive interaction must be mediated somehow through the muscle fibres. The drop in efficacy takes the same form: lower quantal content without any clear-cut change in postsynaptic sensitivity to transmitter.

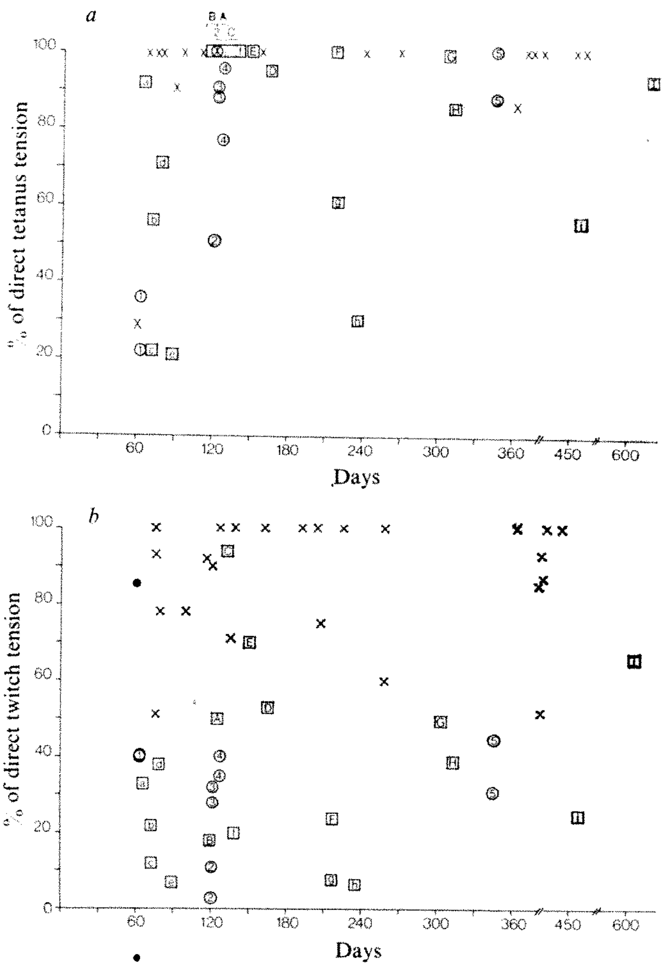
Sartorius muscles were transplanted to the lymph sac beneath the skin of the back of 3–4-inch bullfrogs (*Rana catesbeiana*) and sutured at normal rest length to the fascia of the back. One or two somatic motor nerves (spinal nerves IV and/or V) were implanted into the denervated sartorius muscle. These are mixed nerves, but those fibres forming junctions had conduction velocities characteristic of fast nerves. At different times after innervation muscles were removed to a bath of 15–18° normal frog Ringers, where measurements were made of synaptic potentials and of the contraction tensions elicited by a single stimulus (twitch) and short trains of 30–40 s^{-1} stimuli (tetanus).

When a single foreign somatic motor nerve was implanted into the muscle, it rapidly innervated most or all of the fibres in the muscle (Fig. 1a). Within 2 months, tetanic stimulation of the nerve produced 90–100% of the maximal tension that could be elicited by direct stimulation of the muscle with an electrode applied across the pelvic end (98% ± 5% s.d., $n = 17$ at innervation times greater than 2 months). Maximal direct tension is taken to be that produced when all fibres are contracting.

When two spinal nerves were implanted simultaneously, both nerves seemed to innervate most fibres in the muscle; that is, each nerve was capable of driving the muscle approximately equally well, and in most cases both elicited tetanic tensions within 80% of the maximal direct tetanus tension. Even when one nerve was implanted two or more months before the other, and would be expected to have innervated virtually all of the muscle fibres, the second was frequently able to drive many or all fibres as well (*a,d,f*). In other cases the second nerve was less effective (*b,g,h,i*), but succeeded in innervating many fibres which had presumably already been innervated by the first. The least effective nerves, *c* and *e*, had been implanted for relatively short times. In all cases the first nerve retained effective innervation on essentially all muscle fibres as judged by tetanic stimulation. The interpretation of these tension results as representing extensive dual innervating might be incorrect if strong contraction of one population of fibres could have mechanically induced antidromic action potentials in terminals of the other nerve, innervating a different population. However, no activity could be recorded on one nerve while tetanically stimulating the other, or while strongly stretching the muscle. Moreover, as will be described below, intracellular recording did show double innervation in a large fraction of the fibres.

These results indicate that the presence of one foreign nerve innervating a muscle fibre does not preclude innervation by a second. However, if synaptic effectiveness is judged by the ratio of indirect–direct twitch tension there is evidence for mutual synaptic repression (Fig. 1*b*). A

Fig. 1 Success of innervation by foreign nerves innervating transplanted sartorius muscles, as judged by measurements of the tetanic (*a*) and twitch (*b*) tensions. The tension produced by each nerve, calculated as a percentage of the maximum tension elicited by direct stimulation of the muscle, is plotted at different durations of innervation. ×, indicate success of innervation in singly reinnervated muscles. The encircled numbers represent 5 experiments in which 2 foreign nerves were implanted simultaneously. The letters inside the squares denote data from 9 experiments in which 1 nerve (capital letters) was implanted 2–3 months before the other (lower case letters).



single stimulus to either of two nerves implanted simultaneously caused only 20–50% of the fibres to twitch (mean=31±14% s.d., $n=10$). This was in marked contrast to the effectiveness of a single nerve, which, when implanted alone for 75 d or more (crosses in Fig. 1*b*), caused twitches in 70–100% of the fibres (88±16% s.d., $n=24$). The effectiveness of a nerve in producing a twitch was reduced even if it was implanted 2–3 months before the second, and was presumably able to drive virtually all of the fibres above threshold at the time when the second nerve was implanted. The proportion of fibres twitching to stimulation of the first nerve was 51±24% in nine preparations, far less than if it had been implanted alone. The proportion twitching to the second nerve was generally smaller (19±12% s.d., $n=9$). That a high proportion of junctions had subthreshold responses was also evidenced by the large tetanus–twitch ratio for each of a pair of nerves (average 6.22±4.3 s.d., $n=27$) compared with single reinnervated muscles (2.4±1, $n=21$).

Fibres that were strongly innervated by one nerve tended to be only weakly innervated by the other. Even when one nerve was implanted months before the other, each nerve seemed to have its own separate 'twitch field'. This could be seen both on visual inspection and from the observation that twitch tensions summated with little indication of overlapping innervation. Measuring overlap of functional innervation as the sum of the twitch (or tetanus) tensions to each nerve, minus the tension when both were stimulated simultaneously, divided by the smaller of the individual tensions[16,17], there was usually no more than 10–20% tension overlap of twitch populations, compared with 85–100% overlap of the populations driven by tetanic stimulation (Table 1). The arithmetic sum of the tensions produced by single stimulation of each of the two nerves, implanted simultaneously or at 2–3-month intervals (mean 67±25%, $n=14$), was approximately the same as the twitch tension to simultaneous stimulation of the two nerves (64±23%, $n=14$) and both values were clearly smaller than the average twitch tension of singly innervated muscles (88±16%, $n=24$).

As these tension measurements would predict, a large proportion of the EPPs recorded in doubly innervated muscles were subthreshold to a single stimulus. Moreover, the two nerves apparently seldom form terminals close together on muscle fibres. In approximately 80% of the fibres studied to date in which an EPP was found to stimulation of one nerve (170 of 215 fibres), the other nerve did not produce a recordable EPP at that site. The second nerve either elicited an action potential at some distant point or gave no apparent response. Figure 2*a* is a characteristic example of this group of muscle fibres. In the 20% of the fibres in which EPPs to both nerves could be seen at the same site they usually differed conspicuously in time course and amplitude, although the larger was not always the one with the fastest time course. In less than 5% of fibres were both EPPs of comparable time course, suggesting that they could have arisen close together. We conclude that on most fibres the nerves end at considerable distance from one another, and that one nerve tends to produce a much stronger synaptic response than the other.

Table 1 Overlap of innervation by pairs of nerves

Preparation	Duration of innervation	Overlap % (twitch)	Overlap % (tetanus)
1	64, 64	16	30
2	121, 121	0	100
3	113, 113	15	85
4	126, 126	10	95
5	356, 356	12	100
A, a	125, 65	100	100
B, b	118, 72	0	100
C, c	132, 72	50	100
D, d	166, 79	13	90
E, e	50, 188	0	100
F, f	218, 139	8	100
G, g	307, 217	6	88
H, h	313, 237	0	85
I, i	620, 458	0	85

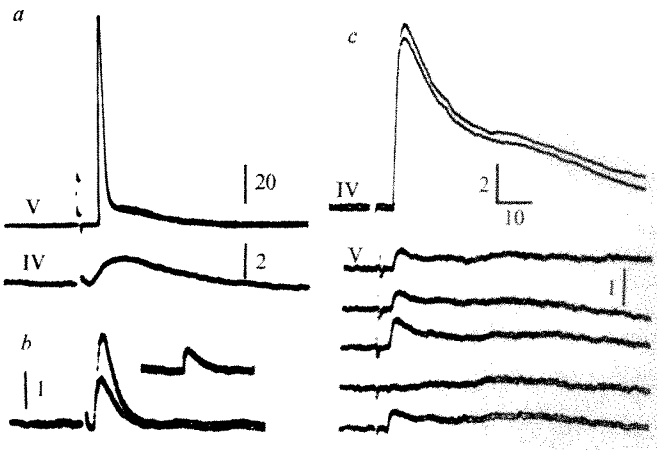


Fig. 2 EPPs from doubly-innervated muscles, all recorded in normal frog Ringer. *a*, Characteristic records from a fibre in a muscle innervated 118 days by both the IV and V spinal roots. At this recording site, nerve IV produced a small EPP, nerve V elicited a spike at some distant point in the fibre. *b*, From the same preparation, low quantal content EPP responses to two nerve V stimuli, compared with a miniature EPP from the same recording site. *c*, EPP responses to stimulation of nerve IV (superimposed top traces) and nerve V (lower series of traces) from a single recording site in a preparation innervated 320 d by nerve V, 259 d by nerve IV. Nerve IV elicited a large subthreshold EPP, nerve V a very low quantal content response that occasionally showed failures. Calibrations are in mV and ms.

EPPs from the weaker synapses (for example, Fig. 2*c*, lower traces) were typically subthreshold in normal frog ringer and showed quantal contents of 10 or less, judged by the amplitude variations of the EPPs and the ratio of average EPP to mean mEPP amplitude. This is in contrast to the EPPs at normal sartorious junctions[18] or to those in singly reinnervated muscles, where virtually all are suprathreshold with quantal contents of 75–100 or more. The dominant synapses on doubly innervated fibres were usually, but not always, suprathreshold also. Their quantal contents, therefore, probably ranged from well above to just below the number required to exceed threshold (approximately 40–50). At the weaker synapses, mEPPs were infrequent, but had rise times and mean amplitudes (range 0.3–1 mV) characteristic of control neuromuscular junctions. Figure 2*b* shows an example. The reduction in synaptic efficacy was thus primarily due to a change in amount of transmitter released, without any evidence for a change in postsynaptic sensitivity. Repression of foreign neuromuscular junctions in salamander limb reportedly takes the same form[9,12]. The reason for the lower-than-normal quantal content at certain synapses is not known but it apparently results from the influence of the other nerve innervating that fibre, and this influence can be exerted at such a distance that it must be mediated somehow through the muscle fibre.

Although the interaction observed here was between two foreign nerves rather than between the 'correct' (original) nerve and a foreign nerve, similar mechanisms may prevail in preparations showing more specificity. Indeed, although Scott[6] has concluded that there is no displacement of a foreign nerve by the original nerve in fish extraocular muscles, she too saw a diminution of effectiveness of both foreign and original nerves, stimulated tetanically, compared with a single reinnervating nerve.

It will clearly be of interest to learn more about the nature of the competitive interaction, the morphological distribution of axons from the two nerves, the interrelationships of their terminals with each other and with old endplate structures, the mechanism(s) whereby one loses influence when a second nerve grows into the muscle, and the degree to which an original (appropriate) nerve is favoured in the competition.

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Is there retrograde axonal transport of tetanus toxin in both α and γ fibres?

IT has been demonstrated by means of histoautoradiography that tetanus toxin, after injection into a muscle, reaches motoneurones in the spinal cord by retrograde axonal transport[1-3]. It was until now not known whether the labelled motor axons and motoneurones belong to the α, the γ, or to both α and γ populations. We now present evidence that tetanus toxin is selectively transported in α motor neurones.

In two cats, ^{125}I-labelled tetanus toxin was injected into one gastrocnemius muscle (4 and 5 μg toxin per kg body weight respectively; the toxin was labelled according to the method of Habermann[4,5], and contained 2 μCi and 4,500 mouse MLD. One cat MLD was equal to 5.4 μg toxin/kg body weight). Three days later the cats were anaesthetised with Nembutal, a laminectomy was performed and the ventral roots L7 and S1 were ligated on the toxin side. The cats survived for another 8½ and 3¼ hours respectively, during which time they were given intensive care[6]. The ligated roots were then excised and immersed in Karnovsky's fixative. Paraffin sections (8 μm) were cut proximal and distal to the ligature and submitted to histoautoradiography using Ilford G5 emulsion. The autoradiographs were developed with Kodak D 19b developer after 71 d and 81 d of exposure at 4 °C. Outer myelin sheath circumferences were measured at a magnification of 800 with a Kontron Manual Optic Point Counting Device (MOP/AM 01). Fibre size histograms were constructed for a representative sample of all fibres (labelled and unlabelled), and for all the labelled fibres, in each of three ventral roots. An example is given in Fig. 1. Two populations of fibres were found, with peaks at around 5 μm and 14 μm diameter. These values are very similar to those described previously for the γ and α populations of cat gastrocnemius motor fibres[7].

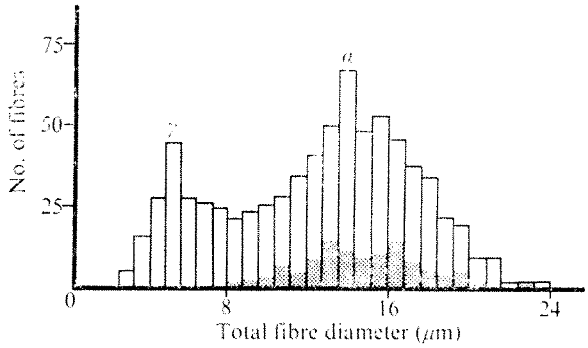

Fig. 1 Fibre size histogram from one fascicle of the ventral root L7 in a cat after injection of ^{125}I-labelled tetanus toxin into the ipsilateral gastrocnemius muscle. Open = labelled and unlabelled fibres ($n = 742$), stippled = labelled fibres ($n = 106$). n = Number of fibres measured. Total number of fibres in fascicle, ~1,500. Total number of labelled fibres, 106.

The accumulation of radioactivity at the ligature would be expected to reveal the presence even of very small amounts of intra-axonal label. Even in these conditions, radioactivity in the ventral roots was found only in fibres which, from their size, seem to belong to the α population[7].

This result is an interesting contrast to the findings of Strick *et al.*[8] who injected horseradish peroxidase into cat gastrocnemius muscle and found γ-motoneurones even more intensely labelled than α-motoneurones. Further studies are needed into the mechanism responsible for the apparent lack of tetanus toxin in the γ axons. A barrier function of structural elements of the muscle spindle or a specific feature of the γ-fibre terminal must be taken into consideration.

Moreover, the increase of γ bias in local tetanus described by Takano *et al.*[9,10] cannot be explained by assuming that tetanus toxin accumulates in γ motoneurones by intra-axonal ascent in γ-motor fibres.

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Modifications of nitrogen-fixing algae in lichen symbioses

THE factors responsible for the establishment of lichen symbioses involving nitrogen-fixing blue-green algae are poorly understood[1,2] and it has not been clear how the algae are modified to remain in association, virtually ceasing to grow and yet fixing N_2 at high rates. We report here that in the lichen genus *Peltigera* morphological, physiological and biochemical modifications of the algae may all be involved in this process.

Comparison of symbiotic and free-living *Nostoc* spp. by light and electron microscopy showed that with *P. aphthosa*, which also contains a green alga (*Coccomyxa* sp.) and a fungus (an ascomycete) as symbiotic partners, the heterocyst frequency of the symbiotic *Nostoc* was 21% while that of the free-living *Nostoc* was 4–5%. The symbiotic algae of *P. canina* and *P. polydactyla*, which have a fungus only as partner, showed no increase in heterocyst frequency (remaining at 4–5% when symbiotic), probably because without another photosynthetic partner overall algal metabolism could not be sustained if more than a fifth of the cells were converted to non-photosynthesising heterocysts. The nitrogenase activity (acetylene reduction[3]) of the symbiotic alga of *P. canina* likewise resembled that of the free-living alga (6–10 nmol C_2H_4 per μg chlorophyll *a* per h) but the nitrogenase activity of the symbiotic alga of *P. aphthosa* (21 nmol C_2H_4 per μg chlorophyll *a* per h) was higher than that of the free-living phycobiont[4,5] and could be accounted for in part at least by the increased frequency of heterocysts. There was also evidence that, in spite of the sustained nitrogenase activity, the symbiotic blue-green alga of each lichen was N-depleted. Thus, compared with free-living algae which accumulate large numbers of cyanophycin granules in post-log-phase growth[6,7], the symbiotic phycobionts showed no large scale deposition of such N reserves.

Physiological differences between free-living and symbiotic algae of *P. canina* were noted when the effect of combined N was investigated. Thus Table 1 shows that, as with other free-living algae[8], NH_3 rapidly inhibited nitrogenase activity by the free-living alga, but had substantially less effect on the nitrogenase activity of the symbiotic alga, although an excess of NH_3 was available. Added NO_3^- did not inhibit nitrogenase activity by the symbiotic alga but partially inhibited that of the free-living alga. That is, in symbiotic algae but not in the free-living algae there is a mechanism which alleviates the inhibitory effect of combined N on nitrogenase.

The activities of important NH_3-assimilating enzymes in free-living and symbiotic algae were then examined. Attention was paid to glutamine synthetase (GS) which may be involved in the regulation of nitrogenase synthesis in heterotrophic bacteria[9,10] and which is the key NH_3-assimilating enzyme in heterocystous blue-green algae[11], to glutamic dehydrogenase (GDH), the primary NH_3-assimilating enzyme in certain eukaryotes, including fungi[12], and to alanine dehydrogenase (ADH) and glutamate-aspartate-aminotransferase (GOT) which occur both in eukaryotic and prokaryotic algae. As Table 2 shows, when the *Nostoc* of *P. canina* was grown free from the lichen, GS activity was particularly high, GDH activity was negligible and GOT and ADH activities were appreciable. In the symbiotic alga, however, GS activity was reduced by 94%, GOT and ADH activities also decreased, and low GDH activities were measurable. In the intact lichen thallus which is mainly fungal hyphae, GDH activity was very high and GS activity was negligible. Possibly the low activities of GDH associated with the symbiotic algae were due to contaminating fungal hyphae in this fraction. In the cephalodia of *P. aphthosa* which consist of *Nostoc* cells and intertwined fungal hyphae, GS activity was much lower than in the remainder of the thallus, indicative of low GS activity in the blue-green algae, but cephalodial GDH activity was much higher than in the remainder of the thallus. Thus, when the blue-green algae develop symbiotically a substantial decrease in activity of their key NH_3-assimilating enzyme GS occurs and in the absence of effective levels of GDH this may result in N-limitation of the algae, in an inability of the algae to grow normally, and in a reduction of the effects of NH_3 and NO_3^- on nitrogenase activity. It is also possible that the low activities of GS, directly or indirectly, are important in the sustenance of a particularly active nitrogenase in such symbiotic algae.

Table 1 Effect of NH_4Cl and KNO_3 on acetylene reduction by disks and by the isolated *Nostoc* of *Peltigera canina*

Concentration of NH_4Cl or KNO_3 (mM)	C_2H_2 reduction after 24 h (% original rate)			
	NH_4Cl		KNO_3	
	Disks	*Nostoc*	Disks	*Nostoc*
0	107	110	107	113
1	58	3	95	42
2	54	3	97	51

The material (disks, 20 × 1.0 cm in diameter, in 50 ml of culture medium[17]) or 50 ml of log phase *Nostoc* were incubated with stirring at 3000 lx and 20 °C for 24 h in the presence of various concentrations of combined N. Acetylene reduction was assayed immediately before addition of the combined N and 24 h later. The level of exogenous combined N (refs 18, 19) did not fall below 0.5 mM and 1.4 mM in the 1-mM and 2-mM cultures, respectively. The initial activities observed were: disks, 7.4 nmol C_2H_4 per µg chlorophyll *a* per h; free-living *Nostoc*, 9.4 nmol C_2H_4 per µg chlorophyll *a* per h. Each value is the mean of triplicate determinations.

To localise NADP-dependent GDH activity cytochemically, sections of *P. canina* thalli and of *P. aphthosa* cephalodia were stained with blue tetrazolium (British Drug Houses, Poole, 0.25 mg ml^{-1}) in the presence of the GDH substrates NADP$^+$ (1 mg ml^{-1}) and glutamate (20 mg ml^{-1}). With *P. canina*, activity was highest in the fungal hyphae closest to the blue-green algae, whereas in *P. aphthosa* cephalodia,

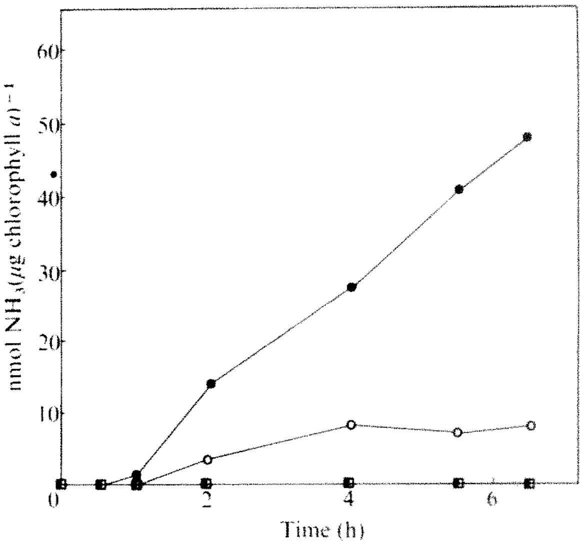

Fig. 1 Time course of production of extracellular NH_3 by 1.0-cm diameter disks of *Peltigera canina* incubated in air + digitonin (●), $A/O_2/CO_2$ (77.96:22.00:0.04, v/v) + digitonin (○), air (■), and $A/O_2/CO_2$ (□). In each experiment 30 disks were incubated with gentle shaking in 50 ml of N-free culture medium[17] at 3,000 lx and 25 °C. NH_3 was determined according to Solorzano[18].

Table 2 Activities of NH_3-assimilating enzymes of *Peltigera aphthosa*, *Peltigera canina* and of the free-living *Nostoc* of *P. canina*

Lichen	Material	Enzyme activities (nmol per mg protein per min) GS	GDH (NADP⁺-dependent)	ADH	GOT
P. canina	Intact thallus	0.8	201.7	3.8	41.0
	Symbiotic *Nostoc*	4.1	2.4	5.5	16.8
	Free-living *Nostoc*	72.4	ND	22.9	40.4
P. aphthosa	Intact thallus	25.7	90.2	—	58.4
	Excised cephalodia	4.5	401.1	—	20.2
	Thallus—cephalodia	17.5	34.8	—	47.0

Peltigera canina and *P. aphthosa* were collected in the field and maintained in the laboratory on N-free medium at 18 °C and 3,000 lx for 24 h before use. The symbiotic *Nostoc* was separated from the remainder of the *P. canina* thallus by the method of Millbank[20]. The free-living phycobiont of *P. canina*, provided by Dr J. W. Millbank, was grown in N-free medium in continuous culture at 25 °C and 3,000 lx. Before enzyme assay the material was washed in 50 mM Tris-HCl buffer, *p*H 7.5, containing 10 mM β-mercaptoethanol and 5 mM ethylene diaminetetraacetic acid. After homogenisation of the lichen material, the homogenates, and the free-living algae, were passed through a French pressure cell at 16,000 pounds per square inch. The extracts were then centrifuged at 35,000*g* for 20 min and the supernatant was passed through Sephadex G-25 to remove low molecular weight compounds then assayed for enzymic activity. The glutamine synthetase (EC 6.3.1.2) biosynthetic assay was that of Kingdon *et al.*[21]. Glutamate dehydrogenase (EC 1.4.1.2.) was estimated by following the oxidation of NADPH at 340 nm in a reaction mixture (3.0 ml final volume): 300 μmol Tris-HCl buffer, *p*H 7.5; 0.3 μmol NADPH; 10 μmol 2-oxoglutarate; 400 μmol NH_4Cl. The glutamate–aspartate–aminotransferase (EC 2.6.1.1.2) and alanine dehydrogenase (EC 1.4.1.1) assays were those of Rowell and Stewart[22]. NAD⁺-dependent GDH was also measured in the above samples using the assay described above with the substitution of NAD⁺ and NADH for NADP⁺ and NADPH. Activity was detected only in the intact thallus (3.8 nmol per mg protein per min) and in the excised cephalodia (4.6 nmol mg per protein per min) of *P. aphthosa*; ND, not detectable; —, not tested.

hyphae surrounding the algae stained heavily but the algae were unstained. The fungal hyphae did not stain in the absence of GDH substrates. Thus, if the N fixed by the symbiotic algae is excreted as NH_3, as would be expected if the primary route of NH_3 assimilation by the algae is blocked, this NH_3 could be assimilated by the fungal GDH.

Finally, we investigated whether the symbiotic *Nostoc* of *P. canina* excreted NH_3. First, disks of *P. canina* were incubated under $A/O_2/CO_2$ (77.96 : 22.00 : 0.04, v/v) or air in the presence and absence of 0.01% (w/v) digitonin, which affects fungal membrane permeability and depresses metabolite assimilation by the symbiotic fungus[13]. Figure 1 shows that during 6.5 h in N_2, but not in its absence, NH_3 was liberated into the medium when digitonin was present. No NH_3 was excreted in the absence of digitonin, and digitonin did not affect nitrogenase activity during the experiment.

Next, *P. canina* disks were exposed to $^{15}N_2$ in the presence of digitonin and the labelling of the extracellular NH_3 and other nitrogenous products was noted. As Table 3 shows, at the end of the experiment the ^{15}N labelling of the extracellular NH_3 was higher than that of the lichen disks and of the organic extracellular N, 50% of the total ^{15}N fixed remained in the disks and, of the remaining ^{15}N incorporated, 50% was released extracellularly as organic N and 50% as extracellular NH_3. Thus as with the symbiotic algae of *Blasia pusilla*[14] and *Azolla*[15], much of the newly fixed N was excreted as NH_3.

Table 3 Incorporation of ^{15}N from $^{15}N_2$ into disks, extracellular NH_3 and extracellular organic N of digitonin-treated *Peltigera canina*

Material	Total N present (mg)	^{15}N-labelling of material (atom % excess ^{15}N)	% of total fixed N in each sample
Disks	70	0.022	50
Extracellular organic N	3.9	0.205	25
Extracellular NH_3	1.1	0.727	25

350 1.0-cm diameter disks of *Peltigera canina* were washed in N-free culture medium[17] for 6 h then incubated in $A/O_2/CO_2$ (77.96:22.00:0.04, v/v) in N-free medium for 12 h. The gas phase was then replaced by $N_2/A/O_2/CO_2$ (10:67.96:22.00:0.04, v/v), the ^{15}N-enrichment of the N_2 being 44 atom % ^{15}N. (All ^{15}N assays were carried out by mass spectrometry using an Associated Electrical Industries MS20 mass spectrometer.) Digitonin (0.01 %, w/v) was also added at 0 time. After 21 h at 1,000 lx and 25 °C the total nitrogen and ^{15}N-labelling of the disks, extracellular NH_3 and extracellular organic nitrogen were determined[23]. The ^{15}N-label of the gas phase did not change over the experimental period. The experiment was run in duplicate.

The above modifications of the symbiotic algae seem to depend on the eukaryotic partner(s) since, as in *Blasia pusilla*[14,16], when the blue-green alga is incubated free from its partners, it reverts to the morphology, physiology and biochemistry of typical free-living forms. Further work is necessary to show whether the eukaryotes produce specific effectors which regulate GS and possibly other NH_3-assimilating enzymes in the cyanophyte, or whether the changes are due solely to internal regulation by the cyanophyte of its metabolism as a result of the presence of the eukaryotes.

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Model for the regulation of nitrate assimilation

NITRATE assimilation is one of the two major biological processes by which inorganic nitrogen is converted to ammonia and thence to organic nitrogen. Photosynthetic organisms, with the possible exception of some blue-green algae and plants that have a symbiotic association with nitrogen-fixing bacteria, derive most of their nitrogen from nitrate[1]. The rate-controlling and regulated step in the process of nitrate assimilation seems to be the conversion of nitrate to nitrite, catalysed by the enzyme nitrate reductase[2]. There is a good correlation between the activity of this enzyme and the yield of grain protein in several cereal crops[3-5]. Nitrate arising from chemical fertilisers and industrial wastes is also a major factor contributing to the growth of algae and other microorganisms, leading to the eutrophication of lakes and streams. Thus, the control of nitrate assimilation can be important from the standpoint of both agricultural productivity and water resource management.

In this communication we propose a model (Fig. 1) for the regulation of nitrate assimilation in which CO_2 fixation and nitrate assimilation are coordinately controlled by the intracellular ratio of $[O_2]/[CO_2]$. This model accounts for the observed effects of O_2, CO_2 and light on nitrate assimilation and provides a rationale for the *in vivo* regulation of nitrate reductase by cyanide.

The nitrate reductase from *Chlorella* and several other organisms can exist in either an active or in an inactive form (see ref. 6). *In vitro* experiments with purified nitrate reductase from *Chlorella vulgaris* showed that two components are required for the rapid and reversible inactivation of nitrate reductase[7]. The most potent combination of effectors is the physiological reductant NADH, and cyanide Cyanide reacts stoichiometrically with the NADH-reduced enzyme to produce a stable enzyme–cyanide complex having a dissociation constant of about 10^{-10} M (ref. 8). These results indicated that very low concentrations of cyanide are sufficient to convert the active enzyme to an inactive form. The conversion of the inactive form to the active form by treatment with an oxidant is accompanied by the release of bound cyanide[8]. Nitrate reductase can be inactivated *in vivo* by brief incubation of the cells with ammonia[9] The *in vivo* inactivated nitrate reductase isolated from such cells contains a stoichiometric amount of bound cyanide that is likewise released on conversion of the inactive nitrate reductase to the active form[9] These results suggested that cyanide is a physiological regulator of nitrate reductase activity.

Pistorius *et al.*[10] have shown that the *in vivo* inactivation of *Chlorella* nitrate reductase is stimulated by O_2 and inhibited by CO_2 and nitrate. Both the effect of O_2 and that of CO_2/nitrate are light dependent[10]. O_2 also inhibits photosynthetic CO_2 fixation[11]. Ribulose 1,5-diphosphate (RuDP) carboxylase can function either as an oxygenase or as a carboxylase[12,13]. Lorimer and Andrews[14] proposed that in the reaction of RuDP with the enzyme, an enolate anion of RuDP is generated which would be susceptible to attack by either CO_2 or O_2. CO_2 fixation could therefore be directly regulated by the ratio of $[O_2]/[CO_2]$. As indicated in the

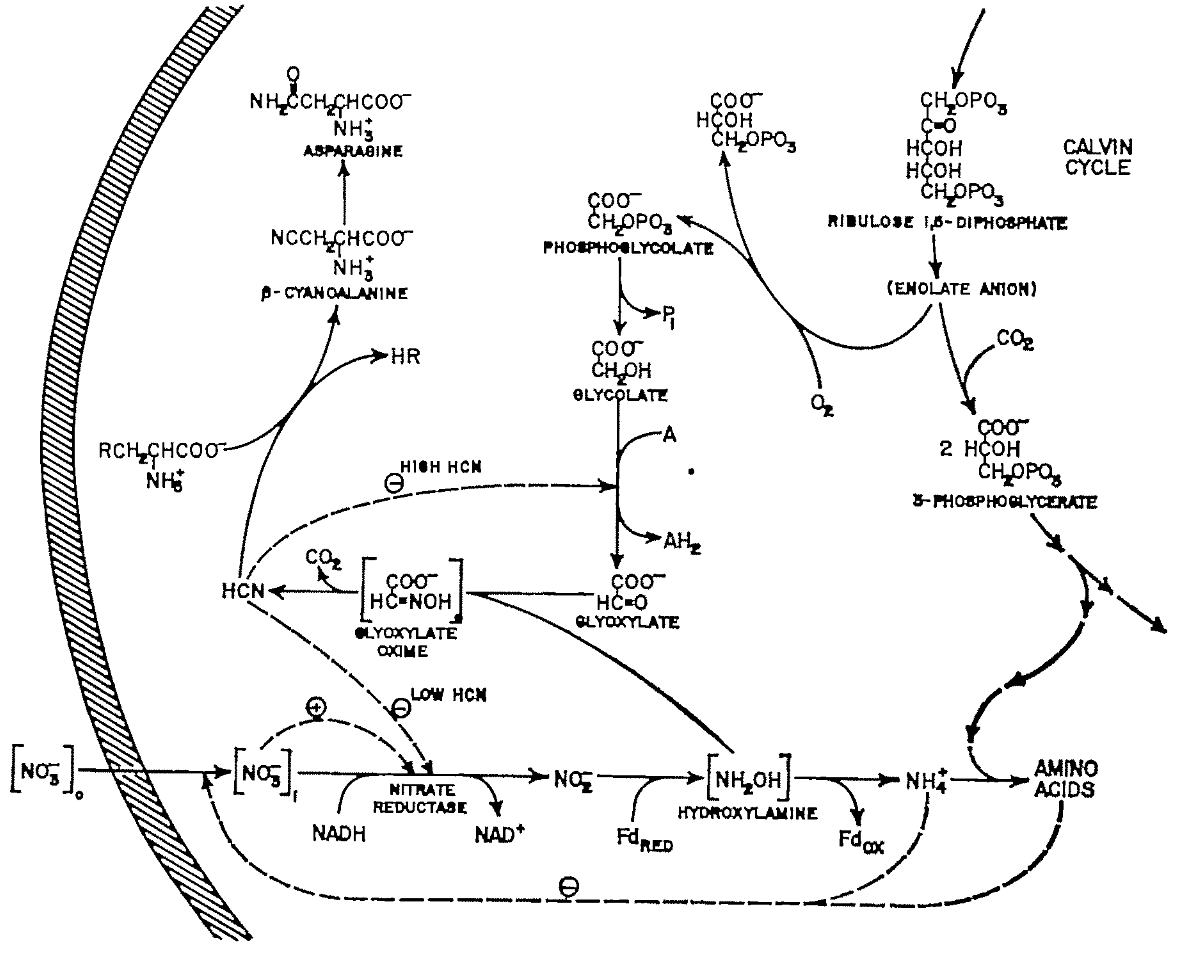

Fig. 1 Model for the regulation of nitrate assimilation. Solid lines represent metabolic conversions. Dashed lines represent inhibition (–) or activation (+) reactions.

model, phosphoglycolate, one of the products of the oxygenase reaction, can be converted to glycolate and thence to glyoxylate[15]. In green algae the oxidation of glycolate to glyoxylate is catalysed by a cyanide-sensitive glycolate dehydrogenase[16]. The physiological hydrogen acceptor for this reaction is not known.

Loussaert and Hageman have recently reported that hydroxylamine is a product of *in vitro* nitrite reduction in the presence of ammonia[17]. A central feature of our proposed model is that hydroxylamine reacts with glyoxylate which is then converted to cyanide and CO_2. This proposal is supported by experiments showing that sonicated extracts[18] of *Chlorella vulgaris* catalyse the formation of cyanide from hydroxylamine and glyoxylate. These reactions were carried out at room temperature in sealed Conway diffusion dishes that contained 0.5 ml of 0.5 N NaOH in the centre well and 2.0 ml of reaction mixture in the outer well. The complete reaction mixture contained 40 mM HEPES NaOH (*p*H 7.5), 5 mM sodium glyoxylate, 5 mM hydroxylamine and 1 mM $MnCl_2$. The reaction was started by the addition of cell extract and was terminated at various time intervals by the addition of 0.2 ml of 50% trichloroacetic acid to the reaction mixture. The dishes were immediately resealed and incubated for an additional 30 min at room temperature to allow complete diffusion of the HCN to the alkali trap. The amount of cyanide in the centre well was then measured by the method of Guilbault and Kramer[19]. The rate of cyanide formation was about 1 nmol min^{-1} mg^{-1}. The activity of nitrate reductase (after activation[7]) in these extracts was about 150 nmol NO_3 reduced min^{-1} mg^{-1}. No significant formation of cyanide was observed in the following controls: (1) absence of hydroxylamine or glyoxylate, (2) with heat-inactivated extracts, or (3) ammonia substituted for hydroxylamine in the complete reaction mixture. The rate of cyanide formation was essentially constant over a 60-min incubation period, was directly proportional to the amount of extract added to the reaction mixture and was stimulated by Mn^{2+}. This stimulation by Mn^{2+} is consistant with the observation that the formation of inactive nitrate reductase, *in vivo*, is impaired in Mn^{2+}-deficient cells[10]. Like nitrate reductase, the enzyme which catalyses the formation of cyanide from hydroxylamine and glyoxylate is found in the supernatant after centrifugation at 100,000*g* for 1 h.

Only very low levels of cyanide ($\sim 10^{-9}$ M) would suffice for the reversible inactivation of nitrate reductase[8]. The concentration of HCN required for the inactivation of nitrate reductase is several orders of magnitude smaller than that which would inhibit respiration[8,20]. The formation of high levels of cyanide could be prevented by the feedback inhibition of glycolate dehydrogenase at higher concentrations of cyanide ($\sim 10^{-6}$ M) (ref. 16). This aspect of the model is also supported by the observation that *Chlorella* cells excrete glycolate at high light intensity, high O_2 tension and low CO_2 tension[21], the same conditions that cause maximum reversible inactivation of nitrate reductase[10]. Added HCN mimics these effects of high O_2 tension[22], suggesting that the cyanide formed internally when cells are illuminated in the presence of high $[O_2]$ causes an accumulation of glycolate.

Excess cyanide could be removed by a reaction sequence in which cyanide is converted to the β-carboxamide of asparagine. Exogenous cyanide is converted to the β-carboxamide group by seedlings of a variety of plants[23]. Fowden and Bell[24] have also shown that seedlings and *Chlorella* convert $H^{14}CN$ to β-cyanoalanine which in turn is converted to asparagine or to the peptide γ-glutamyl-β-cyanoalanine. The feedback regulation of cyanide formation and its removal by the above 'scavenger' pathway would prevent the accumulation of cyanide and the inhibition of other vital metabolic processes such as respiration.

An additional feature of the model is the inhibition of nitrate uptake by ammonia and/or amino acids. A nitrate-uptake system that is independent of nitrate reductase and that is inhibited by ammonia or amino acids has been demonstrated in cultured tobacco cells[25] and in *Neurospora crassa*[26].

A rapid *in vivo* activation of nitrate reductase, independent of *de novo* enzyme synthesis has heretofore not been demonstrated unequivocally. Nitrate, in conditions of high light intensity, high $[CO_2]$ and absence of ammonia causes an apparent *in vivo* conversion of the inactive form of nitrate reductase to the active form[9,10]. Cycloheximide partially inhibits this *in vivo* 'reactivation' of nitrate reductase by nitrate[10], suggesting that the reactivation may be at least partially dependent on *de novo* protein synthesis. Inhibitors of protein synthesis may, however, affect the intracellular concentrations of amino acids thereby inhibiting the nitrate-uptake system[25,26]. This might explain the apparent inhibition of the *in vivo* activation of nitrate reductase by cycloheximide.

It has been demonstrated that when tungsten is substituted for molybdenum in the growth medium, a tungsten

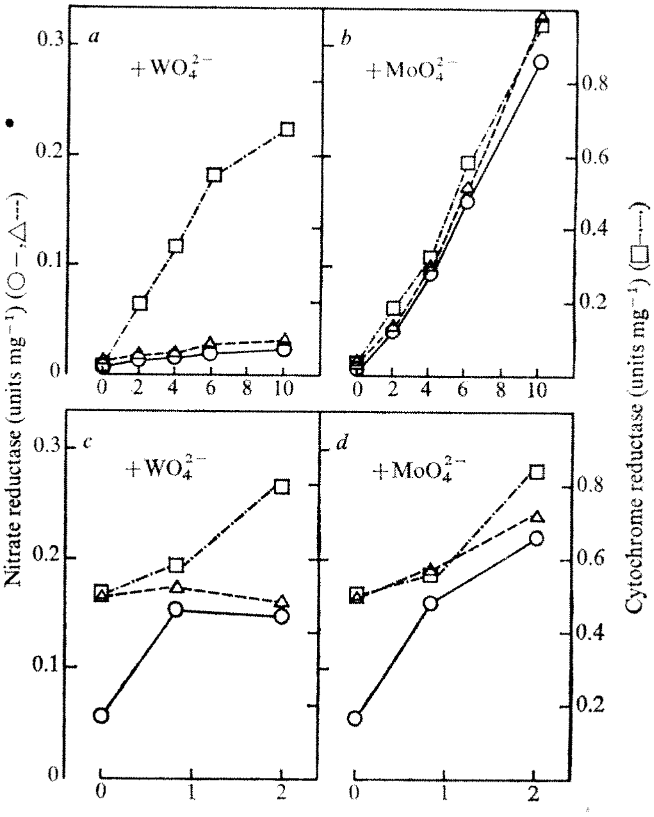

Fig. 2 Nitrate-induced synthesis and *in vivo* activation of nitrate reductase by nitrate. For *a* and *b*, *Chlorella vulgaris* cells were cultured in an ammonium growth medium[29] for 4 d and then were collected and transferred to growth medium containing 10 mM KNO_3 and either 10 μM Na_2WO_4 (*a*) or 10 μM Na_2MoO_4 (*b*). Aliquots of these cell cultures were withdrawn at the indicated intervals, harvested and disrupted by sonication in 10 mM HEPES (*p*H 7.5) and 2 mM KNO_3. The sonicated extract was centrifuged for 10 min at 25,000*g* (4 °C). The supernatant was assayed for NADH–cytochrome *c* reductase activity (□-·-·-·-□) and NADH-nitrate reductase activity before (○———○) and after (△- - - -△) a 2 min incubation with 2 mM potassium ferricyanide to convert the inactive form of nitrate reductase to the active form[7]. Activity is expressed as units (μmol min^{-1}) per mg of protein. The conditions were the same for *c* and *d* except that nitrate-grown cells were preincubated in the ammonium growth medium for 2 h before transfer to growth medium containing 10 mM KNO_3 and either 10 μM Na_2WO_4 (*c*) or 10 μM Na_2MoO_4 (*d*). This short preincubation with ammonium causes an *in vivo* inactivation of nitrate reductase[8,30] in contrast to the disappearance of nitrate reductase and associated cytochrome *c* reductase as occurs on long term cultivation in ammonium-containing medium (*a* and *b*).

analogue of nitrate reductase is formed which has NADH-dehydrogenase (cytochrome *c* reductase) but no nitrate-reducing activity[27,28]. Thus, in the presence of tungstate and absence of molybdate, any new nitrate reductase formed would be expected to have cytochrome reductase activity but no nitrate-reducing activity. By contrast, any increase in nitrate-reducing activity due to the *in vivo* activation of inactive nitrate reductase should be unaffected by tungstate. Therefore, with the use of tungstate, it should be possible to distinguish unequivocally between *de novo* enzyme synthesis and *in vivo* activation of nitrate reductase.

When *Chlorella* cells are exposed to ammonium for long periods (>24 h), the total amount of nitrate reductase, including the associated cytochrome *c* reductase activity, falls to <5% of the original level[29]. When such cells were transferred to a nitrate medium containing tungstate (Fig. 2*a*), there was a normal development of cytochrome *c* reductase activity (see Fig. 2*b*) but no significant development of nitrate reducing activity. Thus, enzyme synthesised in the presence of tungstate has no nitrate-reducing activity. For the experiment shown in Fig. 2*c* and *d*, *Chlorella* cells were exposed to ammonium for 2 h. This short treatment with ammonium causes an *in vivo* inactivation of nitrate reductase[8,30], that is there is no significant change in cytochrome *c* reductase and total nitrate reductase activities but there is a decrease in % active nitrate reductase. When these cells were then transferred to a nitrate medium containing tungstate (Fig 2*c*), there was a normal development of nitrate-reducing activity (measured before activation) over the first 45 min (see Fig. 2*d*). Further development of nitrate-reducing activity, which presumably reflects *de novo* enzyme synthesis, was inhibited by tungstate. These results indicate that the *in vivo* activation of nitrate reductase is independent of *de novo* enzyme synthesis.

Although not indicated in the proposed model, light would have an important role in this regulatory mechanism since ATP and NADPH derived from the light reactions of photosynthesis would be required for the generation of RuDP, the substrate for the reactions catalysed by RuDP carboxylase/oxygenase. As would be predicted from our model, the *in vivo* inactivation and activation of nitrate reductase are both stimulated by light[10]. Also of possible relevance to the proposed model are recent results of Lips and associates[31–33] which suggest that enzymes of CO_2 fixation (RuDP carboxylase), photorespiration (glycolate oxidase) and nitrate assimilation (nitrate reductase) are associated with microbody-like organelles which attach themselves to chloroplasts during illumination.

Since CO_2 fixation by the Calvin cycle is the ultimate source of carbon skeletons for the fixation of ammonia produced by assimilatory nitrate reduction, little or no fixation of ammonia could occur in the absence of CO_2. It is reasonable, therefore, that these two processes be coordinately regulated. When the rate of CO_2 fixation is low, the accumulation of potentially toxic intermediates of nitrate assimilation such as nitrite, hydroxylamine, and ammonia would be prevented. When the rate of CO_2 fixation is high, the formation of sufficient ammonia for the synthesis of organic compounds would be ensured. We propose that this coordinate regulation of CO_2 fixation and nitrate assimilation can be accomplished by the reactions outlined in our model.

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Membrane localised gibberellins A_9 and A_4 in wheat chloroplasts

GIBBERELLINS help to regulate plant growth and development, but their mechanism of action is still not clear[1]. Attempts to correlate the levels of solvent-extractable gibberellins in plants with specific developmental events have shed little light on these problems. Turnover rate is one variable of possible physiological importance not accounted for when steady state levels of extractable hormone are measured. Another relevant factor is the way in which the hormones are sequestered or compartmented within the cell; this may reflect both how the hormone acts and is controlled and how it can be extracted. Prompted by recent evidence that gibberellins are released from wheat[2] and barley[3] etioplasts *in vitro* by red light, we investigated whether gibberellins are associated with membranes in wheat chloroplasts. We found that gibberellins (principally GA_9 and GA_4) can be extracted from chloroplast membrane preparations using novel extraction procedures. Quantities found were much greater than the amounts of gibberellins previously extracted from the vegetative tissues of any plant species.

The non-ionic detergent Triton X100 (ref. 4) was used to disperse membranes in chloroplast preparations obtained from the first leaves of 8-d-old seedlings of *Triticum aestivum* variety Kolibri raised under long photoperiods in a heated greenhouse. Leaves in 10-g lots were ground in a mortar and pestle lined with a 50-μm mesh nylon cloth for 2 min at 2 °C in 100 ml of a modified Honda[5] medium at *p*H 7.2 (9% sucrose; 25 mM potassium-Tricine buffer; 8 mM 2-mercaptoethanol; 5 mM magnesium acetate; Dextran-15; (50 mg ml^{-1}); Ficoll (25 mg ml^{-1}); bovine serum albumin (1 mg ml^{-1}). Material not retained by the mesh was centrifuged at 1,000*g* for 10 min and the supernatant was discarded. Figure 1*a* and *b* shows that about 1,000 times more gibberellin activity of low polarity (R_f 0.5–0.7) was present in Triton-extracted than in methanol-extracted chloroplast preparations. A more polar fraction (R_f 0.1–0.2) was prepresent at relatively low and approximately equal levels in the two extracts. Figure 1*c* shows that only slightly more gibberellin activity was found when the pellet was disrupted by sonication before methanol extraction than when the intact preparation was extracted with methanol directly. The gibberellin activity detected in the methanol extracts was comparable with the activity found when an equivalent weight of leaves was extracted directly with 80% methanol and subjected to the same purification procedure.

Most of the gibberellins so far identified in higher plants have been obtained from immature seeds, because the very low levels hitherto found in vegetative tissues have made identification extremely difficult. The levels of the less polar gibberellins extracted from crude chloroplast preparations with Triton are similar to the gibberellin levels found in methanolic extracts of immature seeds. Hence we were able to identify the gibberellins extracted by Triton without recourse to complex purification procedures. After the usual initial purification steps (Fig. 1), the bulk of gibberellin activity could be resolved into two major, relatively non-polar fractions using silicic acid partition column chromatography[8]. The less polar of these fractions was methylated with ethereal diazomethane and subjected to combined gas chromatography–mass spectrometry (GCMS). Mass spectra were recorded at 3 s per mass decade using an AEI MS30 mass spectrometer with a DS50 data system. Ions of mass 330 (M^+) 298 270 and 271, characteristic of methyl GA_9, were detected at the retention time of authentic methyl GA_9 using the DS50 cross scan facility. GA_9 was identified unequivocally when an exceptionally clean mass spectrum was obtained from a Triton extract subjected to an additional purification step in which the methylated extract underwent Gelman instant thin-layer chromatography[9] (ITLC) in benzene : petroleum ether (boiling point 60–80 °C) (3 : 1 v/v). The zone corresponding in R_f with authentic methyl GA_9 was eluted with ethyl acetate before GCMS.

After the same procedures but with petroleum ether : diethyl ether (7 : 3, v/v) as the ITLC solvent, the more polar fraction from the partition columns was trimethylsilylated after ITLC and, on GCMS, yielded a mass spectrum identical with that of authentic methyl trimethylsilyl GA_4. These identifications will be fully documented elsewhere. None of the gibberellins in wheat plants have been identified before.

We were able routinely to use gas chromatography–single ion current monitoring (GC–SICM) to determine the gibberellin content of partially purified extracts as described in the legend to Table 1. Determined thus, the levels of GA_9 and GA_4 in Triton and methanol extracts of crude chloroplast preparations were similar to the levels measured by bioassay.

To check whether the Triton extractable gibberellins were associated primarily with the chloroplasts, 1,000 *g* crude chloroplast pellets obtained in the usual way were resuspended in 20 ml of extraction medium and eluted with more medium through a 38×3 cm column of loosely packed Sephadex G-50[5]. The first green band to elute from the column (1.5 V_0) consisted almost entirely of intact chloroplasts as seen with phase contrast and electron microscopy. Chloroplasts prepared thus contain per unit chlorophyll the same levels of methanol and Triton-extractable GA_9 as the crude 1,000*g* pellet. That the bulk of the gibberellins are released only by detergent treatment, and not by sonication, suggests an intimate association with the chloroplast membranes. Further evidence in support of this hypothesis was obtained as follows. 1,000*g* pellets were either resuspended in the usual medium and then sonicated or resuspended in the usual medium without sucrose to lyse the chloroplasts osmotically. The disrupted chloroplasts were centrifuged at 20,000*g* for 20 min and the pelleted membranes were extracted with Triton or methanol, treated as usual. The gibberellins extracted with Triton were found by GC-SCIM and by bioassay to be in

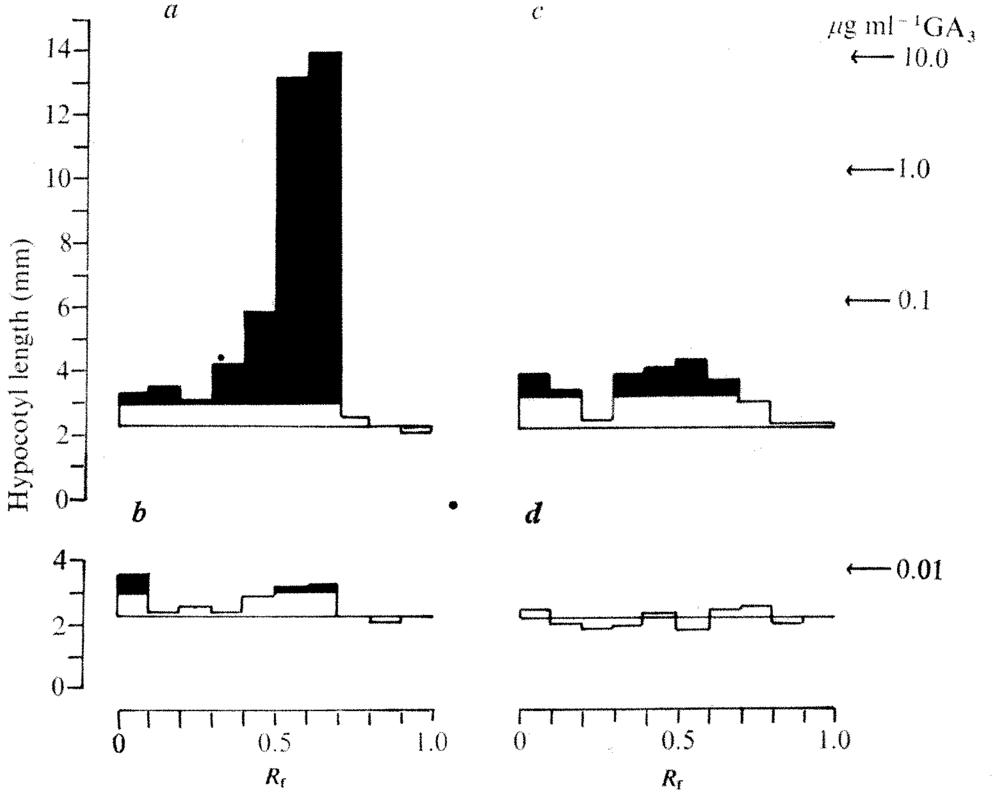

Fig. 1 Gibberellin activity in extracts of the crude 1,000*g* pellet derived from 10 g fresh weight leaf samples. *a*, Pellet resuspended in 50 ml of 2% Triton X100 buffered at *p*H 8.0 in 25 mM potassium Tricine. *b*, Pellet resuspended in 100 ml of methanol : water at *p*H 8.0 (4 : 1, v/v). *c*, Pellet resuspended in 20 ml of grinding medium, disrupted by three 15-s bursts from an MSE 150 W sonicator (nominal frequency 20 kHz), diluted with 80 ml methanol and, after 1 h, centrifuged at 40,000*g* for 1 h and the supernatant taken for further processing. The 40,000*g* pellet from (*c*) resuspended in 50 ml of 2% Triton X100 at *p*H 8.0. All extracts were subjected to a standard initial purification procedure. Methanol was removed under reduced pressure on a rotary evaporator at 35 °C. Extracts were diluted to 200 ml with distilled water, adjusted to *p*H 8.0 and passed through a 2×10 cm column of Dowex IXI-100 formate; the column was washed with a further 200 ml water at *p*H 8.0. Gibberellins were eluted from the resin with 400 ml of ethanol : 1.0 M formic acid (4 : 1 v/v). The eluate was reduced to a small volume of aqueous solution on a rotary evaporator, diluted with 100 ml of 0.1 M Na_2HPO_4, adjusted to *p*H 8.0 and stirred for 1 h with 20 g of insoluble polyvinyl pyrollidone (PVP)[6]. After filtration the extract was adjusted to *p*H 3.0 and partioned three times against half its volume of ethyl acetate. Bulked ethyl acetate extracts were dried with anhydrous magnesium sulphate and the solvent was removed on a rotary evaporator. For bioassay, purified extracts dissolved in methanol were applied to 10-cm wide sheets of Whatman No. 1 chromatography paper. Chromatograms were developed in *iso*-propanol : 0.880 : ammonia : water (10 : 1 : 1, v/v/v), cut into strips corresponding to 0.1 -R_f units and the strips bioassayed directly using the lettuce hypocotyl test[7].

Table 1 Yields of GA$_9$ and GA$_4$ from chloroplast membrane preparations

	Assay method			
Extraction procedure	GC–SICM		Bioassay (μg GA$_9$ equivalents)	
	GA$_9$	GA$_4$	GA$_9$	GA$_4$
Triton X100 extracts (μg mg^{-1} chlorophyll)	2.9	0.9	2.1	0.5
(μg g^{-1} fresh weight leaves (adjusted for membrane recoveries))	7.7	2.4	5.9	1.4
80% Methanol extracts (μg mg^{-1} chlorophyll)	0.005	0.001	0.004	0.001
(μg g^{-1} fresh weight leaves (adjusted for membrane recoveries))	0.013	0.004	0.01	0.003

Crude chloroplast pellets from 10 g fresh weight of leaves were resuspended in 20 ml extraction medium and sonicated as before. Sonicated preparations were centrifuged at 20,000*g* for 20 min and the pelleted material was extracted with Triton or aqueous methanol. The extracts were partially purified (Fig. 1) and for GC–SICM the ethyl acetate extracts were methylated with ethereal diazomethane. The methylated extracts were subjected to ITLC using benzene–petroleum ether (3 : 1, v/v) as solvent for methyl GA$_9$ or petroleum ether–diethyl ether (7 : 3, v/v) as solvent for methyl GA$_4$; zones corresponding in R_f to those of authentic methyl GA$_9$ or methyl GA$_4$ were eluted with ethyl acetate. The ethyl acetate was removed on a rotary evaporator and the extract was redissolved in 2 ml of acetone. Methyl-GA$_9$ or methyl-GA$_4$ content was measured by injecting 1 μl of the acetone extract on to a glass column (182 cm × 0.4 mm inner diameter) packed with 2% OV-1 coated on Gas Chrom Q (100–120 mesh) fitted to a Pye 104 gas chromatograph coupled through a silicone rubber membrane separator to an AEI MS 30 mass spectrometer. Samples were chromatographed isothermally at 228 °C with 40 ml min^{-1} helium carrier gas. The MS 30 was set to monitor current at an *m/e* value of 298 for GA$_9$ or 224 for GA$_4$ with 1,000 resolving power using authentic methyl GA$_9$ or methyl GA$_4$ as reference compounds. High frequency noise was eliminated by a filter circuit adjusted to give the minimum peak distortion and the selected ion current was recorded with a Servoscribe ls pen recorder. The total ion current was recorded on a second Servoscribe. Authentic GA$_9$ added to 50 ml 2% Triton X100 could be recovered after these procedures in yields of > 90%. For bioassay, acidic ethyl acetate extracts were line loaded on to 10 × 20-cm thin-layer chromatography plates spread with 250 μm silica gel. Plates were developed in ethyl acetate–chloroform–acetic acid (15 : 10 : 1 v/v) and dried overnight over a fan. The silica gel was removed in bands corresponding to 0.1 R_f values and bioassayed directly using the lettuce hypocotyl test[7]. Authentic GA$_9$ moved in the solvent system used to R_f 0.85–0.9 and GA$_4$ to 0.65–0.7. Chlorophyll was determined according to Arnon[10].

the membrane pellets (Table 1), which per unit of chlorophyll contained similar amounts of gibberellin as intact chloroplasts. Extracted separately with methanol, the membrane pellet and supernatant yielded approximately equal amounts of gibberellin; combined pellet–supernatant gibberellin content was similar to that of intact chloroplast preparations extracted by methanol.

Circumstantial evidence suggests that the Triton-extractable gibberellins are localised within the chloroplast membranes in a specific manner, rather than simply partitioned into the membrane lipids. The gibberellins are not extracted by chloroform : methanol mixtures which are reported to delipidate membranes completely[11]. And they are not extracted from sonicated chloroplast preparations by 8 M urea or by 1 mM EDTA, which would be expected respectively to release components linked to the periphery of the membrane by hydrogen bonds or by metallic cations[12]. One possibility is that the Triton-extractable gibberellins are intimately associated with non-polar proteins or other molecules located within rather than on the periphery of the membranes. This might explain not only why the Triton-extractable gibberellins are not extracted with methanol but also why methanol renders them not extractable with Triton. The detergent failed, for example, to extract gibberellins from the insoluble residue which remained when the chloroplasts were sonicated and extracted for 1 h with 80% methanol and then centrifuged at 40,000*g* for 1 h (Fig. 1*d*). Furthermore, the amounts of gibberellin extracted by methanol were not increased when Triton was added to unfractionated methanolic extracts or when methanol and Triton were added together. Possibly, methanolic extraction denatures or alters the proteins or other molecules with which the Triton-extractable gibberellins are intimately associated, in such a way that the gibberellins are rendered not extractable even by Triton.

Clearly, there seem to be at least two fractions of extractable gibberellins compartmented within the chloroplast membranes—one released from the membranes by methanol and another released by Triton. Whether these fractions belong to the same physiological pool, part of which is not efficiently extracted by organic solvents, or whether the two fractions are physiologically distinct needs to be determined.

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Removal of 5-bromo-2-deoxyuridine incorporated in DNA of regenerating rat liver

THERE are many systems available for the study of *in vitro* DNA repair, including autoradiography[1], inhibition of *de novo* DNA synthesis by hydroxyurea (HU)[2] and use of radioactive[3] or cold 5-bromo-2-deoxyuridine (BUdR) as substrates, followed by density gradient separation[4] or photoirradiation[5] at 313 nm. There are a few methods available for *in vivo* studies of DNA repair[6] based on indirect techniques involving the use of DNA alkaline sucrose gradient centrifugation, or on the use of HU and gel filtration in tissues with low mitotic rhythm (unpublished work). Use of BUdR in DNA labelling does not give satisfactory results *in vivo*, and autoradiography is impractical. We have examined the hypothesis that BUdR, once incorporated in newly-synthesised DNA, can be recognised as a steric encumbrance and so be removed by nuclear DNA repairing enzymes. This removal does occur, and forms the basis of a reliable method for measuring DNA repair in tissues with high DNA-synthetic activity.

Female Wistar rats (in groups of five) weighing 130–150 g underwent partial hepatectomy[7]; 23 h later, that is at the peak of the S phase, a mixture of ^{3}H-thymidine (TdR) and ^{14}C-BUdR (18.5 Ci mmol^{-1} and 60 mCi mmol^{-1}, respectively),

Table 1 Removal of ^{14}C-BUdR incorporated in DNA of regenerating rat liver

First experiment	0*	1	2	3	4	8	15
^{3}H/^{14}C ratio	6.52±1.52	6.72±1.41	—	6.30±2.40	—	15.35±2.64‡	17.88±1.18‡
^{3}H-TdR-DNA labelling (d.p.m. mg^{-1})	12,000±850	10,500±646	—	4,805±573	—	3,889±466	3,384±397
^{14}C-BUdR-DNA labelling (d.p.m. mg^{-1})	1,840±221	1,563±188	—	763±91	—	253±30	186±31
Second experiment							
^{3}H/^{14}C ratio	7.57±0.55	9.30±0.90	5.27±0.93	—	13.94±1.91†	21.89±1.78‡	25.65±2.08‡
^{3}H-TdR-DNA labelling (d.p.m. mg^{-1})	12,596±1,512	10,502±1,260	5,600±661	—	4,603±549	4,082±460	3,499±420
^{14}C-BUdR-DNA labelling (d.p.m. mg^{-1})	1,664±140	1,129±136	1,063±128	—	330±40	186±22	136±20

*Zero time values were obtained from a group of rats killed 1 h after i.p. injection of 9.1 μCi ^{3}H-TdR together with 1.3 μCi ^{14}C-BUdR (^{3}H/^{14}C ratio = 7) 23 h post-hepatectomy in female Wistar rats (130–150 g) in groups of five. Animals were killed at various times and radioactivity was counted on duplicated 1.5-mg DNA samples. Data are reported as mean ± s.e.m.

†$0.05 > P > 0.01$.

‡$P < 0.01$.

(Radiochemical Centre, Amersham) was injected intraperitoneally in the ratio ^{3}H/^{14}C=7 (9.1 μCi TdR plus 1.3 μCi BUdR). In the first experiment rats were killed 1 h and 1, 3, 8 and 15 d after injection, while in a second experiment rats were killed 1 h and 1, 2, 4, 8 and 15 d after treatment. The livers were removed and processed separately. DNA was extracted, and purified from the isolated nuclei using a phenol method[8] and radioactivity was determined in 0.4-ml samples of supernatant made 0.2 N $HClO_4$ after centrifugation. Two samples (about 1.5 mg) of DNA extracted from each of the livers were dissolved in distilled water, acidified, filtered on Whatman GF/C glass fibre dishes, washed with ethanol and then ether and dried. Finally, 3 ml methylcellosolve and 10 ml scintillation liquid (0.4% PPO and 0.005% POPOP in toluene) were added to each vial and the double labelling (^{3}H and ^{14}C) was counted in a previously calibrated Intertechnique SL 32 spectrometer, provided with external (^{226}Ra) standardisation.

Table 1 shows removal of ^{14}C-BUdR from DNA in regenerating rat livers; results are expressed both as the mean of the ratios ^{3}H/^{14}C and as the mean of DNA specific activity for each isotope ±s.e.m. Figure 1 shows the variation in time of the DNA specific activity for ^{3}H and ^{14}C (multiplied by 7 to be normalised to ^{3}H) from the second experiment. The finding of a ratio ^{3}H/^{14}C in DNA equal to that of injected TdR/BUdR is casual and due to preferential uptake of TdR, diluted in the cell nucleotide pool. In Fig. 2, liver growth is plotted against time, and the quantity of DNA is expressed both as mg and as mg g^{-1} of tissue. CsCl sedimentations of DNA samples confirmed that ^{3}H and ^{14}C labels were associated with the DNA peaks (1.705 density) in the previously reported ratios.

BUdR removal from DNA began 4 d after initial treatment and stopped during the eighth day, that is, within a period in which DNA synthesis is completed and liver mass and DNA quantity per unit of weight have practically returned to normal. We are not aware of any reports of BUdR removal *in vivo* by a purely physicochemical mechanism and so we consider the observed BUdR removal as an indication of DNA repair. An alternative explanation of the data might be that cells were killed following incorporation of labelled BUdR and TdR, and that released ^{3}H-TdR is preferentially incorporated into DNA. This is unlikely, however, because both BUdR (1.7×10^{-7} M) and TdR (3.8×10^{-9} M) were administered in doses well below toxic levels (10,000-fold and 1,000-fold lower, respectively)[9,10]. The ^{3}H-TdR dose used was 100-fold lower than the mutagenic dose in mouse[11], and the dose given per g body weight

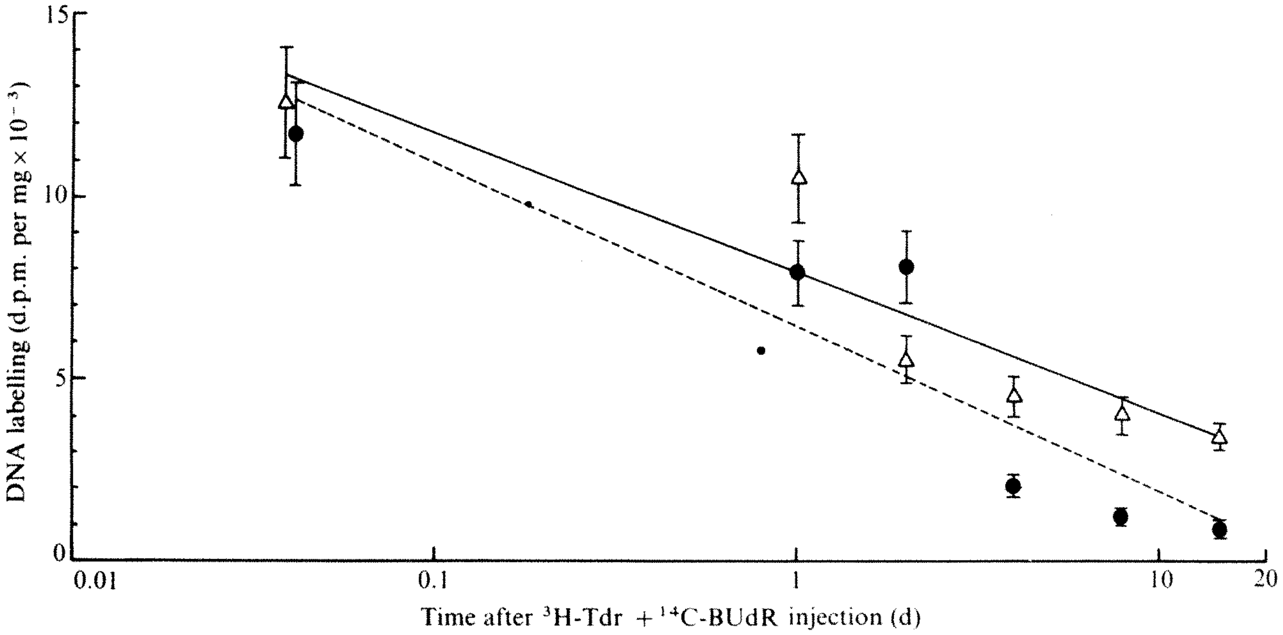

Fig. 1 DNA labelling time course after injection of 9.1 μCi ^{3}H-TdR and 1.3 μCi ^{14}C-BUdR (second experiment) to rats 23 h after hepatectomy (time 0). Data are the mean of duplicate estimations performed on five animals ± s.e.m. ^{3}H regression line: $y = 7851-3885.26x$ ($t = 28.83$). ^{14}C (normalised to ^{3}H) regression line: $y = 6461-4498.79x$ ($t = 38.34$); in both x = time log. Slopes were significantly different at $P < 0.05$, when regression lines were compared.

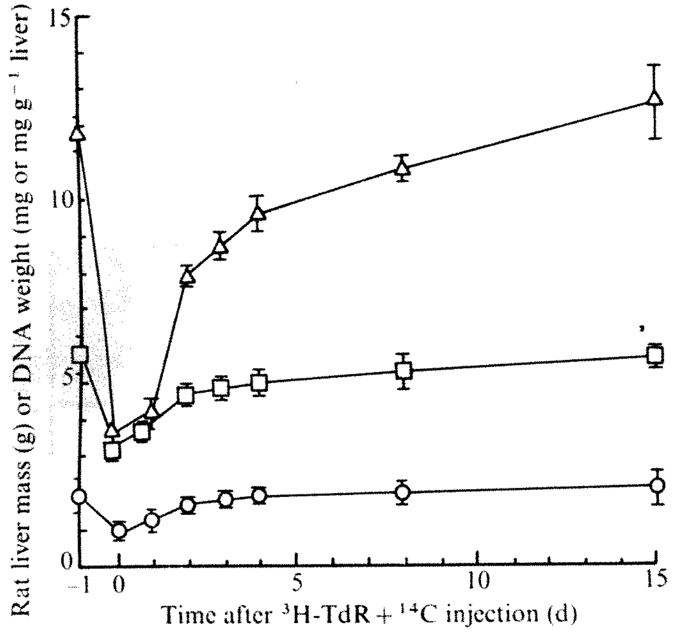

Fig. 2 Time course of rat liver mass as g (□) and DNA weight, both as mg (△) and as mg g^{-1} tissue (○) after partial hepatectomy. Animals were injected with ^{3}H-TdR/^{14}C-BUdR as in Fig. 1, at 23 h after hepatectomy (time 0). Data are the mean five values ± s.e.m.

was sixfold lower than a dose which affects neither rat liver regeneration (as regards liver mass) nor mitotic rhythm and figures[12], in identical conditions. The dose of ^{3}H-TdR we used *in vivo* does not influence cell viability when added to cell culture, an *in vitro* system where physiological removal of radioactive tracer is impossible[10]. On the basis of radioactivity incorporated into DNA, and assuming that there was 10 pg of DNA per cell, there were only 1.6 cell disintegrations within the first 8 d, and even fewer when it is considered that cell division occurs. Variations in ^{3}H/^{14}C ratio occurred when mitotic index was again low. Moreover BUdR is similarly removed in rat spleen cell cultures (our unpublished work) in the presence of excess cold TdR.

It is therefore possible that, when BUdR is inserted into DNA, there is filament breakage similar to that which occurs rapidly *in vitro* after photoirradiation, and that enzymatic repair can follow[13]. BUdR-induced breakage seems to take place more slowly than the radiation-induced process. Removal and the repair processes could follow the molecular mechanism involved in repairing γ-ray-induced damage[5].

The method we describe can be used both to compare repair activity as a function of age in organs with high DNA synthesis and to study the relationship between carcinogenesis and DNA repair in systems, such as regenerating liver, for which data are available on tumour induction by single administrations of *N*-methyl-*N*-nitrosourea[14], dimethylbenz(a)anthracene[15], dimethylnitrosamine[16] and urethane[17].

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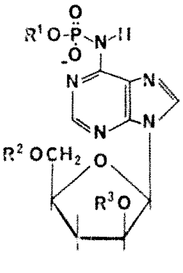
Agrocin 84 is a 6-*N*-phosphoramidate of an adenine nucleotide analogue

We wish to draw attention to the natural occurrence of an extraordinarily selective antibiotic, agrocin 84, which is a 'fraudulent' member (structure I) of the hitherto unreported 6-*N*-phosphoramidate series of adenine nucleotides (structure II), of which 6-*N*-AMP (II, R = H) is the first member.



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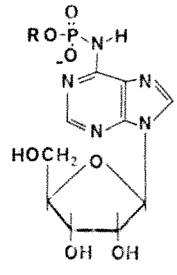

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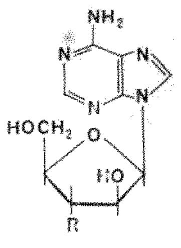

III

Kerr and Htay[1] have shown that the biological control of crown gall, achieved by the inoculation of susceptible plants with the non-pathogenic strain 84 of *Agrobacterium radiobacter*, is due to the production of a bacteriocin by the controlling organism. This bacteriocin, now known as agrocin 84, selectively inhibits the growth of converted pathogenic strains of *A. radiobacter*, while the growth of most non-pathogenic strains is unaffected[2]; this property is utilised in the bioassay, which was originally developed by Stonier[3] for another bacteriocin. Heip *et al.*[4] have outlined a partial purification procedure for agrocin 84 from solid media, but the degree of purification was not established and no structural studies were reported.

We have found that isolation is greatly facilitated by culturing a rough colony variant of strain 84 in a defined liquid medium. After a 10,000-fold purification of the agrocin 84 had been achieved by gradient elution techniques involving charcoal adsorption and anion exchange chromatography on DEAE Sephadex, a correlation between biological activity and the presence of a nucleotide with an absorption spectrum maximum at 264 nm was noted and found to hold for all the chromatographic and electrophoretic systems which have been investigated.

After further purification by electrophoresis on glass-fibre filter paper agrocin 84 has been characterised as an adenine nucleotide with the characteristic spectra shown in Fig. 1*a*. The purified agrocin contained adenine, phosphorus, 3′-deoxy-D-arabinose and D-glucose in the molar ratio of 1:2:1:1. It also seems to contain an as yet unidentified reducing sugar acid, and the presence of other constituents cannot be excluded at the present stage of the purification. An upper limit for the molecular weight of the triethylammonium salt is set at 1,100 ± 100 by an $A_{1\,\mathrm{cm}}^{1\%}$ = 180 and an ε value of 19,860 at 264 nm and neutral *p*H for the purest solid samples obtained to date.

By defining an arbitrary agrocin 84 unit as that amount of agrocin necessary to inhibit totally a zone of radius 1.0 cm within a lawn of pathogenic indicator strain 24, using the

Stonier[3] bioassay, we have found at *p*H 7, that one 264 nm absorbance unit of the purified agrocin 84 contains $8 \pm 1 \times 10^3$ agrocin units. On a molar basis this corresponds to 1.6×10^{11} units mol^{-1}.

The infrared spectrum of agrocin 84 showed the presence of a P–O stretching frequency at 1,225 cm^{-1} and the absence of simple carbonyl groups and the pyrophosphate grouping. The electrophoretic mobility/*p*H profile indicated the absence of any secondary dissociation corresponding to a phosphate monoester.

Glucose and 3′-deoxy-D-arabinose were identified as the main carbohydrate components of an acid hydrolysis of agrocin 84 by chromatographic and electrophoretic comparison with authentic samples; the pentose reference sample was obtained from a crystalline sample of methyl 2,5-di-*O*-acetyl-3-deoxy-β-D-*threo*-pentofuranoside by the procedure of Casini and Goodman[5]. Alkaline hydrolysis (1 M NH_4OH, 100 °C 30 min) of the agrocin 84 produced a crystalline adenine nucleoside containing this unusual pentose, with the typical ultraviolet spectral behaviour ($\varepsilon_{max}^{260.5}$ 14,300, *p*H 12.55; $\varepsilon_{max}^{260.0}$ 14,400, *p*H 6.92; $\varepsilon_{max}^{258.0}$ 14,200, *p*H 1.06) for a 9-substituted adenine nucleoside.

It is now a decade since the corresponding 'fraudulent' nucleoside 9-(3′-deoxy-β-D-*threo*-pentofuranosyl) adenine (structure III, R = H) was first synthesised by Martinez, Lee and Goodman[6] in the course of a cancer chemotherapy programme. Although the original sample is no longer available, the required nucleoside has been synthesised by desulphurising the penultimate intermediate 9-(3-S-ethyl-3-thio-β-D-arabinopentofuranosyl) adenine (structure III, R = CH_3CH_2S–) with Raney Nickel.

The synthetic nucleoside (structure III, R = H) and the nucleoside from the alkaline degradation of agrocin 84 were found to be indistinguishable in all the properties examined, including melting point, mixed melting point, optical rotation, paper and thin-layer chromatographic systems, electrophoretic systems, gas chromatography of the trimethylsilyl derivatives, mass spectra of the nucleoside and its silyl derivative, ultraviolet spectral behaviour, and infrared spectra.

With the establishment of the structure of the nucleoside fragment of agrocin 84 as structure III (R = H), attention was next directed to the nature of the substitution of the nucleoside, which would result in the ultraviolet spectra of the intact agrocin as shown in Fig. 1*a*. Four properties of the agrocin which are particularly relevant, are first, the initial bathochromic shift in the absorption maximum from 264 nm to 270 nm on acidification (Fig. 1*a*); second the relatively rapid hydrolysis of the agrocin in 0.1 M HCl at room temperature to produce a nucleotide with an absorption maximum at 260 nm at neutral *p*H values and whose ultraviolet spectral shape and behaviour are similar to other 'normal' 9-substituted adenine nucleotides; third the extreme thermal lability (110 °C, *p*H 7, 15 min) of agrocin 84 at neutral *p*H to produce a second nucleotide with an absorption maximum at 260 nm; and finally the room temperature hydrolysis of agrocin 84 in alkali (1 M NH_4OH, 25 °C, 3 h) to produce a third nucleotide, which is thermolabile, contains D-glucose, has an absorption maximum at 264 nm, and is similar in ultraviolet spectral behaviour to the intact agrocin. These three nucleotides are readily resolved from agrocin 84 and one another, by electrophoresis in 0.05 M sodium tetraborate buffer at *p*H 9.4.

The reported[7,8] bathochromic shifts for 6-*N*-acylated 9-substituted adenine nucleoside derivative and the known thermolability and acid lability of phosphoramidates led us to postulate the presence of a 6-*N*-phosphoramidate grouping. Model 6-*N*-phosphoramidates of adenosine were therefore synthesised from 2′,3′,5′-tri-*O*-acetyl adenosine[9] and the appropriate phosphate, using dicyclohexylcarbodiimide in pyridine as the condensing agent, under conditions analogous to those described by Tener[10] for the condensation of cyanoethyl phosphate with 2′-deoxyadenosine. Figure 1*b* shows the spectral characteristic of 6-*N*-(cyanoethylphosphoryl) adenosine (structure II, R = $CNCH_2CH_2$–). At *p*H 5.0 the spectral behaviour of the monoprotonated 6-*N*-(hydrogen phosphoryl) adenosine (6-*N*-AMP) (structure II, R = H) with an absorption maximum at 264 nm, ε, 19,500, resembles its cyanoethyl precursor shown in Fig. 1*b*. The non-protonated species of 6-*N*-AMP in 0.1 M sodium hydroxide, shows a shift to 267.5 nm, ε, 19,800.

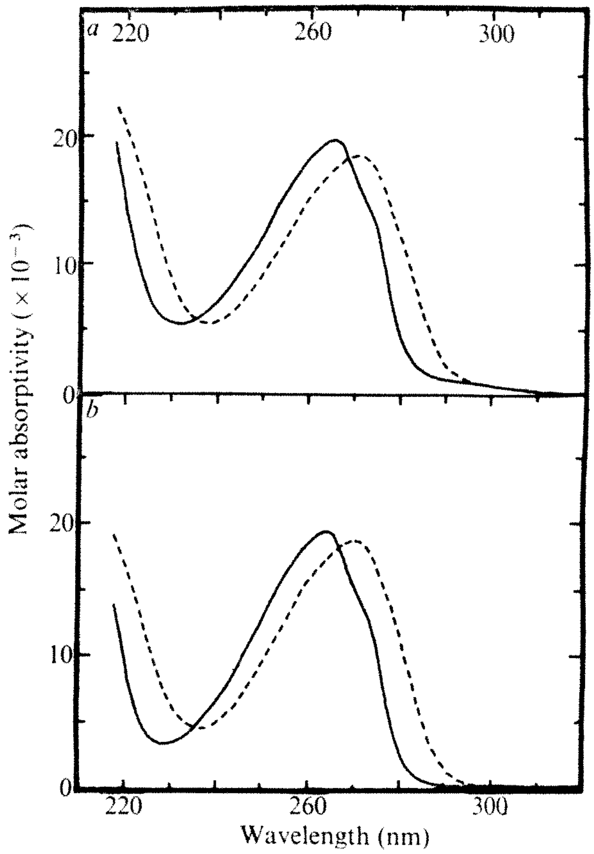

Fig. 1 *a*, Absorption spectrum for agrocin 84 at *p*H 1 (- - -) and 7 (———). *b*, Absorption spectrum for 6-*N*-(cyanoethylphosphoryl) adenosine (structure II, R = $CNCH_2CH_2$–) at *p*H 1(- - -) and 7 (———).

The striking resemblance between the spectra of agrocin 84 in Fig. 1*a* and the 6-*N*-(cyanoethylphosphoryl) adenosine in Fig. 1*b* leaves little doubt that agrocin 84 contains the hitherto unrecognised 6-*N*-phosphoramidate linkage to adenine as shown in structure I, where R^1 is at present considered to contain glucose. R^2 and R^3 represent hydrogen and a second phosphorylated carbohydrate moiety or vice versa.

The current investigation has firmly established the structure of the 'fraudulent' nucleoside fragment and the unique aspect of the phosphoramidate linkage is clearly indicated. If we accept the definition of a bacteriocin as simply 'an antibiotic produced by strains of bacteria and in general active on some other strains of the same species or related species'[11] and do not further restrict it to being 'colicin-like' as mentioned by Reeves[11], then this agrocin 84 should probably be classed as a new type of bacteriocin.

In conclusion, we point out that current attempts to explain all biochemical phenomena involving adenine nucleotides solely in terms of the 2′,3′, and 5′-phosphate esters may be less than adequate and a thorough search for other labile phosphoramidates such as the naturally occurring agrocin 84 and the synthetic 6-*N*-AMP reported here, with their predictably high phosphoryl transfer potentials, should be rewarding.

We thank the Australian Research Grants Committee for support. The award of a Charles John Everard Scholarship to W.P.R. is gratefully acknowledged. The ethylthionucleoside

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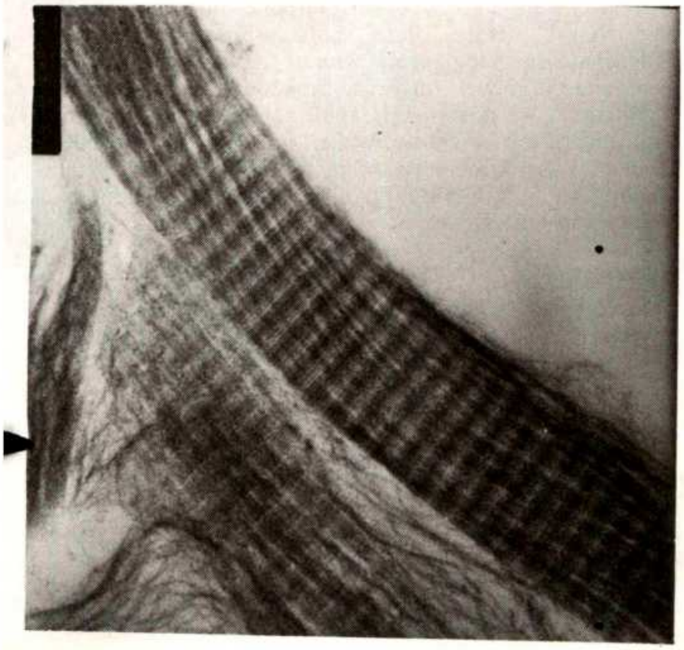
Three-dimensional packing of collagen in bone

Bone collagen, which forms approximately 90% of the organic matrix of bone, differs from rat tail tendon and skin collagen in its solubility properties[1]. Using low-angle X-ray meridional photographs and electron microscopy, however, I have found that the banding pattern of the collagen fibrils of bone is similar to that in other collagenous tissue, but that low-angle X-ray equatorial pattern could not be found.

Many explanations[2,3] have been proposed for the banding pattern of collagen fibrils (Fig. 1) based on the low-angle meridional pattern of X-ray photographs and on electron micrographs. But to describe the packing of tropocollagen molecules within the collagen fibrils in bone, which can be deduced from low-angle equatorial pattern of X-ray photographs, it is necessary to take account of the presence of needle-like mineral particles aligned along the collagen fibrils and the lack of orientation of collagen fibrils with respect to one another.

Fig. 1 Electron micrograph of section of demineralised foetal bone (× 11,000).



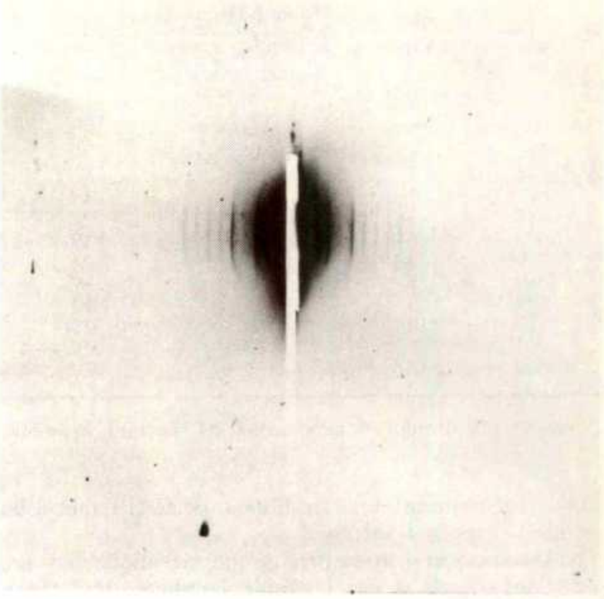


Fig. 2 Low-angle X-ray photograph of demineralised mature bone in water taken at Birkbeck (resolution shows only in one dimension).

Low angle meridional patterns of bone collagen in its mineralised and demineralised form were recorded by using a low-angle X-ray apparatus of the Holmes-Huxley type (mirror and crystal-monochromator)[9] (Fig. 2), but no low-angle equatorial pattern could be found even after 3 d of continual exposure to X irradiation.

The experiment was repeated at DESY (Deutches Elektron-Synchrotron) in Hamburg because synchrotron radiation is a more powerful source of X irradiation, and because in that situation[8] meridional and equatorial patterns can be recorded simultaneously. Low-angle X-ray photographs of calcified turkey tendon (as a reference) and

Fig. 3 Low-angle X-ray photograph of turkey leg tendon (dry) taken at DESY.

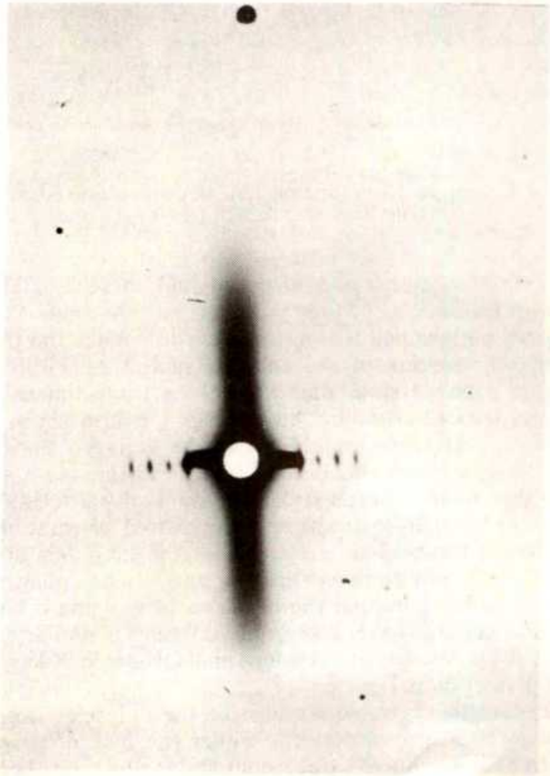


Table 1 Distances and intensities of meridional low-angle X-ray lines (average)

	No.	Bone (synchrotron) (Å)	I	Bone (Birkbeck) (Å)	I	Tendon (synchrotron) (Å)	I
—	1	—		645	S	621	VS
676	2	338	S	343	VS	365	M
678	3	226	VS	222	VS	232	VS
672	4	168	W	162	W	173	S
685	5	135	S	132	S	141	S
672	6	112	W	111	W	117	S
679	7	97	M	95	M	101	W
680	8	85	W	32	W	87	W
675	9	75	M	73	M	78	W
680	10	68	VW	66	VW	70	VW
682	11	62	VW	60	VW	64	VW
660	12	55	VW	54	VW	—	—

I, intensity; S, strong; VS, very strong; M, medium; W, weak; VW, very weak.

demineralised and mineralised human cortical femoral bone were used (Figs 3, 4 and 5).

The distances and intensities of the meridional low-angle X-ray lines (rings) shown in Table 1 indicate that there is little difference between the distances and intensities of the various orders of the low-angle meridional lines (rings) in tendon and bone. The main difference between the X-ray photographs is that in bone the meridional lines are extended to form full circles. Thus there is no low-angle X-ray equatorial pattern and it is not possible to deduce the three-dimensional packing of the collagen molecules within the fibrils or even to state that there is a three-dimensional order, as seen in tendon. The photographs also show that there is a preferred orientation of the collagen fibrils at about 22–23° from the c axis and that the continuous scatter due to the short axis of the mineral particles (Fig. 5) is perpendicular to the zone of preferred orientation of the collagen fibrils. This suggests that the long axis of the mineral particles lies along the long axis of the collagen.

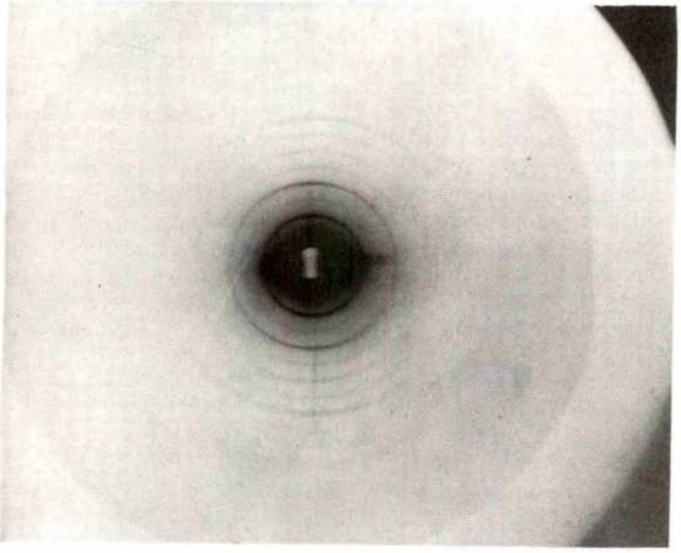


Fig. 4 Low-angle X-ray photograph of demineralised mature bone in water taken at DESY.

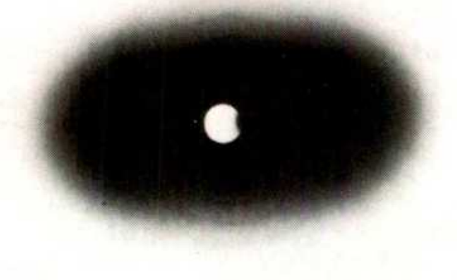


Fig. 5 Low-angle X-ray photograph of mineralised mature bone in water taken at DESY.

These results show that the ultrastructure of the collagen fibrils of bones and other collageneous tissues is similar when examined by electron microscopy and low-angle X-ray diffraction (meridional pattern).

There are two possible reasons for the failure to observe a low-angle equatorial pattern : either the lack of orientation (full rings) conceals the equatorial pattern or there is no equatorial pattern and hence no three-dimensional ordering within a collagen fibril in bone. In either case low-angle X-ray pictures alone cannot show whether three-dimensional packing exists within the collagen fibrils in bone, and the models for rat tail tendon, based on the same technique[4–7] are not directly applicable to bone collagen.

This work was completed while I was at Birkbeck College, London. I thank members of the Department of Crystallography and also EMBO for a fellowship and the use of facilities at DESY.

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matters arising

Energy analysis of wave-power and wind-power systems

Musgrove[1] paints a depressing picture of the energy analysis of wave power but seems to have largely overlooked the literature on its progress[2,3] The model tested by Swift-Hook *et al.*[4] was of amateur construction as I can personally vouch. Since then the generous support of the Department of Industry has allowed developments which point to an optimum duck diameter of about 15 m instead of the 50 m suggested by Denton *et al*[5]. This reduces the displacement to 200 tonnes per metre instead of the 2,000 quoted by Musgrove.

There is a further respect in which Musgrove's analysis is incorrect. He assumes that we intend to use steel, but the high energy content of steel is a strong incentive to use as little of it as possible Concrete requires only one seventh of the energy of steel[6] and it is suitable for much of the structure.

I am keenly aware of the many difficulties facing wave power and so I am hesitant to accept his prediction that (with correct input data) the energy repayment period will be 16 years divided by 70 which is eighty or ninety days.

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[1] Musgrove, P. J , *Nature*, 262, 206 (1976)
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Musgrove replies—In my recent letter[1] I estimated that the energy recovery period of a rocking float wave power system would be about 16 years Salter believes that this may be greatly reduced. In particular he suggests that reducing the diameter from about 50 m to about 15 m will reduce the energy recovery period by a factor of ten; the use of concrete instead of steel will then, he suggests, reduce the energy recovery period by a further factor of 7

My original letter did consider the effect of reducing the diameter of rocking float systems but, as I pointed out, reduced diameter gives reduced efficiency and an increased ratio of structure weight to displacement; the gain is therefore appreciably less than Salter suggests. Moreover, the simple calculation, which in my letter gave an energy recovery period of 16 years, was based on a wave power system sited at Station India, 700 km out in the Atlantic, where the annual average power flux is 77 kW m^{-1}. A more typical location is Seven Stones, off Land's End, where the annual average power flux is 26 kW m^{-1}. At this location a 50 m diameter rocking float system would have an energy recovery period of approximately 47 years, and lack of alignment between the wave power system and the incident waves will tend to increase this figure. Reduced diameter does however give some reduction in the energy recovery period and hence I concluded that overall one could expect to achieve an energy recovery period of about 16 years

Matters arising

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This estimate does assume steel construction, consistent with the assumption, made by Denton *et al.*[2], that the cost per unit displacement of rocking float wave power systems will be comparable with that of existing supertankers. The specific energy content of concrete is, I agree, much lower than that of steel, but no one has yet built a concrete supertanker. I sincerely hope that the use of concrete will allow a seven-fold reduction in the energy recovery period of rocking float wave power systems. However, even if this can be achieved, it should be recognised that wind power systems have the potential to provide as much energy as wave power systems, and present much less formidable development problems.

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[Power outputs for different sizes of installation have been given by Mollison *et al.* in *Nature*, Vol. 263, pp 223–236, September 16, 1976. Ed.]

Use of mechanical barriers instead of guard rows in field experiments

A technique in which vertical strips of Vinyl copolymer foil were used to separate experimental plots in a large field experiment on fruit trees was reported in *Nature* in 1962[1]. The experiment, involving different water and nitrogen regimes, reached a successful conclusion in 1975, and during 1976 an examination was made of the foil and of the behaviour of the roots when they encountered it. The foil was intended to serve as a barrier to root extension across plot boundaries in place of guard rows of trees. This was achieved surprisingly well; roots were contained without any penetration of the foil and remained at the depth at which they had met the barrier and turned.

Since the barriers were laid to a depth of 0.86 m, root exploration downwards might have led to serious poaching from adjacent plots. However, this does not appear to have occurred even after 15 years.

In this experiment the need for 4.4 ha of land with guard trees was reduced to 1.9 ha; this saving of experimental land together with greatly reduced risks of inter-plot variability and the satisfactory containment of treatments and roots at a cost considerably below that of concrete barriers[1] probably afford the technique a wide application.

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Plutonium and americium in fish

PENTREATH and Lovett[1] have presented data on $^{239+240}$Pu/^{238}Pu ratios in plaice which cause us some concern in the light of what we have recently reported on the probability of biological discrimination between different plutonium isotopes[2]. Although not discussed in their report the ratios in different fish tissues from any one composite sample seem to show approximately two- to threefold differences in the uptake behaviour of ^{238}Pu and $^{239+240}$Pu, depending on the time of year. As no consistent trends among the ratios in the various tissues seem evident, we question the significance of these differences. Counting errors associated with the $^{239+240}$Pu measurements run typically about 20% (range 4–47%). The errors for the ^{238}Pu measurements are not reported but undoubtedly are higher due to the lower levels of ^{238}Pu in the samples. Furthermore, where counting error for $^{239+240}$Pu is the least (for example, gut contents), the ratios are more consistent and tend to approach a mean value of 4.4, which is in good agreement with the ratio (4.3) measured in surface sediment[2] collected from the same general area in July 1975. In fact, if the same relative counting errors reported for the $^{239+240}$Pu measurements are assigned to the corresponding computed ^{238}Pu values, comparison of the mean values of the ratios for all tissues from each collection do not indicate differential behaviour between the isotopes. We propose that if the counting errors associated with both the ^{238}Pu and $^{239+240}$Pu measurements were propagated, the resultant errors about the ratios would indicate that the apparent differences in plutonium isotope behaviour in certain tissues suggested by their data are not, in fact, significant.

Furthermore, we question the advisability of computing concentration factors for plutonium and americium in plaice in which only filtered seawater concentrations of the actinides are used. In this case the impression given is that ^{241}Am accumulates in plaice muscle fivefold over that of $^{239+240}$Pu. In another study[3], concentration factors in worms based on total plutonium and ^{241}Am in Windscale sediments indicate that the accumulation of plutonium is favoured over that of ^{241}Am by a factor of two. The discrepancy could be due to a 'species effect'; however, it also may be simply an artefact of the different way in which the concentration factors were computed.

Summation of the monthly discharge data reported by Pentreath and Lovett gives an estimated integrated annual discharge ^{241}Am/plutonium ratio of about 1.4, which is not too unlike either that measured in the sediments[3] (1.7) sampled in July 1975 or those that can be computed from their data for the various fish tissues (especially November 1975) and gut contents. These similarities suggest to us the possibility of a real ^{241}Am/plutonium ratio in water somewhat higher than 0.2 reported for ^{241}Am and $^{239+240}$Pu in their filtered sample. One recent report[5] states that 30% of the $^{239+240}$Pu in their filtered samples. One recent report[4] states that 30% of the $^{239+240}$Pu in Windscale waters is associated with filterable particulates (>0.22 μm). While apparently no comparable published data for ^{241}Am exist, there are indications[5,6] that ^{241}Am may be strongly particulate in seawater. We recognise the limitations involved in determining concentration factors; however, until more information is at hand concerning the bioavailability of both soluble and particulate forms of these elements, we believe that concentration factors based only on one fraction of the total element in water may be artificially high.

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PENTREATH AND LOVETT REPLY—The errors associated with the ratios of $^{239+240}$Pu/^{238}Pu were omitted in order to avoid a discussion on them. Fowler and Beasley are correct in assuming that most of them are not significantly different, as deduced from errors associated with counting. But there were one or two samples which did seem to be different and these are being investigated further. By and large, however, we are generally impressed as to their lack of variance in view of the widely differing ratios which obtain in the Windscale area. Fowler and Beasley argue from the axiom of a constant ratio, assumed to be $\simeq 4.3:1$. In fact the ratio in the Windscale discharge is not accurately known but seems to have declined from $\simeq 20:1$ to $\simeq 4:1$ in the last 10 years as deduced from measurements made on surface sediments[1]. The ratio thus also varies with depth in the sediment, and this variance with depth differs from one area to another. The fish may therefore have been exposed to slightly different seawater ratios and to largely different ratios in ingested material. Because each organ may have a different biological half-time for plutonium, different ratios could be expected in the organs of some fish. This is rather speculative, however, and it would be very difficult to demonstrate in individual fish and could only explain large ratios.

With regard to the comments concerning the calculation of concentration factors, these calculations were made using the only data available at the time—filtrate sea water samples taken from the same area and at the same time as the August fish samples. These data were kindly proffered by a colleague and we thought the calculations made with them would be of interest; we still do. In doing so, however, we wished neither to commend nor condemn the use of filtrate seawater values for the general calculation of concentration factors. There are as many arguments against using total seawater values—such as the variability in particulate matter in shoreline areas—as there are for their use. The views on this subject of one of us (R.J.P.) are discussed at length elsewhere[2]. For the Windscale area the greatest sources of error for plaice are likely to be, as stated in our paper, the variable discharge rate and the movements of the fish into and out of the area close to the discharge pipe.

Finally, with regard to the amounts of ^{241}Am relative to those of plutonium, we remarked that the concentrations of the former may well have been influenced by a large release at the end of 1974. It is of interest that in samples taken in February 1976 the ratio was $>2:1$ in the majority of organs whereas that of the discharge during the previous six months has been $<0.5:1$.

In short, therefore, the principal objective of the paper was to give some indication of the concentrations of plutonium and americium in plaice in comparison with the amounts of these radionuclides discharged. The points raised by Fowler and Beasley were not discussed as we considered it more advisable to obtain further data rather than attempt to over-interpret those few data which we had already obtained.

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reviews

Ethology of ethology

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Growing Points in Ethology. By P. P. G. Bateson and R. A. Hinde. Pp. viii+548. (Cambridge University: Cambridge, 1976.) £16.

MY reaction on perusing this volume was one of optimism for the future of ethology. An appreciable proportion of the eighteen contributions to this symposium, held in June 1975 to celebrate the 25th anniversary of the Madingley Field Station (Cambridge, UK), discuss interesting new ideas and approaches or bring new evidence to bear on areas of current excitement. In comparison with a parallel volume produced by Madingley ethologists and their associates 15 years ago the theoretical arguments are less concerned with semantics and classification, and more with real attempts to seek underlying generalisations. It is also comforting to note that most of the major areas of interest have changed considerably in 15 years. The editors, who have done an excellent job in writing brief discussions to summarise, coordinate, and occasionally criticise the individual essays, have arranged the volume along the lines of Tinbergen's classical 'four questions'—causation, function, evolution and development. There is also a considerable section on 'human ethology'.

In spite of the risk of being accused of chauvinism, I must state that I found two of the most original articles to be those by Dawkins and McFarland, both of whom have tried to develop new approaches to constructing general theories of behaviour Dawkins argues that explanatory principles of the mechanisms of complex behaviour should come at the purely behavioural, rather than physiological level. After the burial of some early ethological models of motivation this sort of approach has not been much developed. But since the complete physiological description of complex behaviour is a distant and perhaps not even fruitful goal, "software" explanations, as Dawkins calls them, are obviously of crucial importance. Dawkins goes on to reason on functional, "neuroeconomic" grounds that evolution will tend to develop hierarchical control mechanisms of behaviour, and that this should be a general principle of behavioural organisation. He also describes various analytical tools for detecting hierarchical properties of overt behaviour.

McFarland also argues for software explanations of behaviour, but from a different standpoint. He suggests that even if one knew all the physiological mechanisms underlying, say, feeding and sexual behaviour in the dog, one would still not be able to look at a dog and predict whether it will eat or copulate next, because this will depend on how the dog balances the two tendencies against one another in making a decision. The decision rules can only be analysed at the behavioural level, and the problem is essentially an ecological one, because the animal will have been designed by evolution to make ecologically sensible (adaptive) decisions. McFarland then outlines a theoretical method for tackling the difficult problem of how a cost–benefit analysis of decision making can be achieved. The aim of the theory is to predict the optimal decision rules, which can then be tested against the actual data. Although this type of approach has been used in the ecological literature on foraging behaviour, this is the first attempt to apply the rationale to many different behaviour patterns at the same time.

The recent surge of interest in kin selection theory, and especially in its role in the evolution of altruistic behaviour, is reflected in the articles by Clutton-Brock and Harvey on primate social groups, and by Bertram on lion prides. The former paper is a useful discussion of aspects of primate social organisation, such as pair bonding, altruism, aggression and sexual dimorphism, in the light of current evolutionary thinking. Bertram takes the analysis of kin selection further (to my knowledge further than any other vertebrate study, with the possible exception of the work on group territorial birds) by calculating the degree of relatedness (r_0) between the various members of a lion pride. Bertram then discusses whether or not kin selection can account for various altruistic acts in the pride. For example, mothers suckle one another's cubs, even though the degree of relatedness between a mother and these cubs is very low, about 0.075. So kin selection is not likely to have played a major role in the evolution of this trait. On the other hand, the observation that males more than females are tolerant of cubs feeding at kills, fits in well with the calculated differences in r_0: 0.31 for males and cubs, and 0.18 between females and an average cub. Clutton-Brock and Harvey discuss an analogous example of dominant Japanese macaques tolerating subordinates according to how closely related they are.

The section on human ethology contains two particularly useful articles (by Hinde and Hinde, and Blurton Jones) in which the interface between ethology and social psychology (in the former) and anthropology (in the latter) are explored. Hinde and Hinde make a convincing case that it is now possible to study on a truly scientific basis the qualitative nature of inter-individual relationships, a problem from which most ethologists would have recoiled in horror ten years ago. Blurton Jones urges that the deductive approach, based on natural selection theory, used for explaining and predicting social organisation in animals, should also be applied to human cultures. Peculiar cultural customs should not be regarded as quirks of chance drift, fascinating and inexplicable, but viewed as ecological adaptations. In this way, he argues, it should be possible to develop a coherent theory of cultural differences.

Returning to my opening comment, this volume shows convincingly that ethology is not, as E. O. Wilson has prophesied, about to meet its death at the hands of neurophysiology and sociobiology. There is still a remarkably exciting atmosphere resulting from the blending together of causal, developmental and functional questions about behaviour, that still characterises ethology. ☐

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Hydrodynamics and water waves

An Introduction to Hydrodynamics and Water Waves. By Bernard Le Méhauté. Pp. viii+323. (Springer: Berlin and New York, 1976.) DM 60.60; $24.80.

AN obvious question to ask about this book is: what are the special contributions which it offers to an already well endowed literature on hydrodynamics and water waves? The declared aim of the book is to provide a text based on a set of lecture notes for engineering students, that introduces the mathematical aspects of fluid mechanics with explanations of physical meaning to help practising engineers to read mathematical texts and keep pace with new developments reported in scientific journals. It is intended, moreover, to have a wide range of application and in particular to appeal to "students of coastal, hydraulic and civil engineering, naval architecture, physical oceanography, marine geology and sedimentology".

Although it inevitably covers much well trodden ground, it fulfils its aims and has much to offer in clarity of explanation and in its orderly presentation of a broad selection of theoretical material. It may be disappointing, however, to those seeking a close contact with current engineering developments.

The text is divided into three main parts concerned, respectively, with the derivation of the basic equations of fluid motion, mathematical treatment of the equations, and water wave theories. There are two appendices, one dealing with wave spectra and associated statistical properties, and the other with problems of scale modelling.

Examples of clarity include well illustrated accounts of the distinctions between streamlines, stream tubes, streak lines and particle paths and of the significance of dilatation, shear and rotation in interpretating the kinematic equations. I specially commend the many well organised tabular summaries of key details and formulae which students will surely find very helpful and which offer convenient working references for practising engineers. Students should also appreciate the selection of illustrative problems at the end of each chapter for which answers are given in the back of the book. Although prominence is given to development of basic theory relevant to a broad treatment of water waves, material is included on flow in porous media and pipes, and on open channel hydraulics. Its claim to appeal to students in a wide range of professional fields therefore seems justified.

Although the book offers a clear and broadly based conceptual framework for the study of theoretical fluid dynamics the degree of contact with current engineering technology may be disappointing to those with a more practical turn of mind. A particular feature, for example, that I find rather frustrating is the almost total absence of cited references. There are lists called "References" at the end of each part but these might more appropriately be headed "Recommended background reading", since there are hardly any explicit references to these works in the text. The reader is therefore repeatedly tantalised by the introduction of complex ideas such as tensors, conformal mapping, ship waves and diffraction theory which are only very briefly discussed without mention of any references for further study.

N. Hogben

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Acetylcholine, the model transmitter

Biology of Cholinergic Function. Edited by A. M. Goldberg and I. Hanin. Pp. xiv+716. (Raven: New York, September 1976.) $42.

BOOKS for biomedical scientists often promise more than they actually provide because the titles of convenience they bear have been conjured up by ingenious editors who wish to confer (on a disparate collection of symposium papers, for instance) a suggestion of cohesiveness and completeness where none exists. It is a pleasure to be able to declare at the very outset that *Biology of Cholinergic Function* escapes this criticism: it really does provide everything—perhaps more than—its title offers. I find it difficult to believe that anyone who buys the book will be disappointed with his purchase, even though his intellectual enrichment will be bought at the cost of economic impoverishment to the tune of £26 at this morning's rate of dollar exchange.

Neurotransmission processes attract a good deal of attention nowadays, and deservedly so, but in the midst of our animated discussions concerning such lively topics as monoamines and mental illness, the reawakening of interest in the possible transmitter roles of ATP, substance P and histamine and the enthusiasm in many quarters in an increasing range of transmitter amino acids, it is easy to ignore acetylcholine, the earliest, the best authenticated and the most pervasive mediator of them all. Cerebral control systems rarely lack cholinergic synapses even though recent attention may seem to have been largely focused on their more immediately glamorous non-cholinergic components. Work on acetylcholine has, however, continued apace during the years when other substances have occupied the centre of the stage; and no worker in, or student of, the field of chemical transmission can afford to ignore it. It is conveniently assembled for him within the substantial covers of this book.

This massive compilation (more than 700 pages) is encyclopaedic in scope with exhaustive and up-to-date surveys of the literature (papers published as recently as 1975 are referenced) and of recent experimental findings. The main body of the text—18 chapters in all—discusses acetylcholine metabolism and actions in vertebrates and invertebrates, at subcellular and at whole organ levels, at peripheral and central sites, in muscle and in nerve. Those who champion the claims of non-cholinergic transmitters would do well to pay particular attention to the chapter that discusses the interaction of cholinergic and non-cholinergic transmitter processes and to the group of four chapters that together form a section on cholinergically linked diseases. The book is enriched for the laboratory worker by the inclusion of two appendicular tables. One of them, nineteen pages long, provides information concerning the acetylcholine content and cholinesterase activity of the tissues. It lists the results of all the studies that have worked their way through into the literature. The other table, only two pages shorter, does the same for the actions of drugs on the acetylcholine system. These tables are described in the preface as compendia of 'cholinergic parameters' but, this semantic monstrosity apart, the literary style of the book as a whole is tolerable if not exactly memorable.

Multiauthor texts are prone to exhibit irregularities in style and in depth of treatment and to suffer from omissions and repetitions that irritate the busy reader. This volume cannot be faulted on these grounds because the twenty-nine contributors worked together as a team under the guidance of editors who clearly took their duties seriously. All twenty-nine authors live in the United States but this does not unfairly bias the book towards American workers and attitudes. I hope that this judgment is not unduly influenced by the fact that my own modest contributions to the subject of the book are not ignored.

James Crossland

James Crossland is Professor of Pharmacology at the University of Nottingham, UK.

PROCEEDINGS QUICKLY

Hormonal Receptors in Digestive Tract Physiology

Proceedings of the First International Symposium on Hormonal Receptors in Digestive Tract Physiology, held in Abbaye de Royaumont, France, 22-24 September 1976

INSERM SYMPOSIUM, No. 3

edited by S. Bonfils, P. Fromageot *and* G. Rosselin

1977 xx + 520 pages US $ 47.95 / Dfl. 120.00
ISBN 0-7204-0618-8

The major features of this book are: the biochemical, immunological and biological characterization of peptide hormones or synthetic analogs with emphasis on gut hormones; study of hormone receptor interaction binding; and the analysis of the cyclic nucleotide system in the intestinal tract, salivary gland, external pancreas and liver.

Hormones and Cell Regulation*

Proceedings of the First INSERM European Symposium on Hormones and Cell Regulation, held at Le Bischenberg, Bischoffsheim, France, 27-30 September, 1976

edited by J. Dumont *and* J. Nunez

1977 240 pages US $ 24.95 / Dfl. 62.00
ISBN 0-7204-0622-6

Leading scientists discuss the most recent developments in the field of hormones and cell regulation, ranging from various types of hormone and neurotransmitter receptors to the regulation of cellular secretions, glucose metabolism and protein synthesis. Further meetings are planned and this book is the first in a series.

HLA Systems - New Aspects

Proceeding of the International Symposium on HLA Systems - New Aspects, Bergamo, Italy, 25-26 September, 1976

edited by G. B. Ferrara

1977 180 pages US $ 22.95 / Dfl. 57.00
ISBN 0-7204-0617-X

Research on HLA has become intensive and this book concentrates on those areas where the most significant developments are taking place, including: the biological and biochemical properties of histo-compatibility antigens; serology and transplantation; and MLC typing in man.

Rheumatoid Arthritis

Cellular Pathology and Pharmacology

Proceedings of the Cambridge Rheumatism Group Symposium on Rheumatoid Arthritis: Cellular Pathology and Pharmacology, held in Cambridge, U.K., 21-23 September, 1976

edited by J. L. Gordon *and* B. L. Hazleman

1977 x + 286 pages US $ 33.75 / Dfl. 84.00
ISBN 0-7204-0621-8

This book provides the most up-to-date review of advances in genetics, immunology, tissue physiology and therapeutics related to rheumatoid arthritis. It will prove invaluable to clinicians who wish to know the current views of this growing area of research.

Regulation of Cell Membrane Activities in Plants

Proceedings of an International Workshop held in Pallanza, Italy, 26-28 August, 1976

edited by E. Marrè *and* O. Ciferri

1977 xii + 334 pages US $ 31.95 / Dfl. 80.00
ISBN 0-7204-0615-3

The papers presented in this volume constitute the first attempt to integrate two of the most important and, until now, relatively separate fields of plant physiology - physiology of transport and hormonology.

Modern Trends in Bacterial Transformation and Transfection

Proceedings of the Third European Meeting on Bacterial Transformation and Transfection, Granada, Spain, August 31 - September 3, 1976

edited by A. Portolès, R. López *and* M. Espinosa

1977 xii + 332 pages US $ 31.95 / Dfl. 80.00
ISBN 0-7204-0608-0

This up-to-date review of molecular genetics covers the following topics: physico-chemical aspects of competence; processing of irreversibly bound DNA in gram-negative and gram-positive bacteria; recombinational events and heterologous transformation; restriction enzymes mapping and cloning; and transformation and clinical aspects.

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PHYSICIANS' HANDBOOK OF NUTRITIONAL SCIENCE (2nd Ptg.) *by* **Roger J. Williams,** *The Univ. of Texas, Austin, Texas. Foreword by* **I. Newton Kugelmass.** A straightforward, sound, conservative and readable account of the crucial facts of nutrition are provided in this book. In the few instances where the author speculates or voices his own opinion, the difference between fact and opinion is made evident. The twelve basic principles of nutritional science are clearly outlined by the author and are reviewed by several prominent nutritional experts. Some of the topics considered are basic principles underlying nutritional science, internal nutrition, prenatal nutrition, biochemical individuality, and problems with environment and its effect on nutrition. *'76, 128 pp., $9.75*

ESSENTIALS OF BACTERIAL AND VIRAL GENETICS *by* **David M. Carlberg,** *California State Univ., Long Beach, California.* Beginning with the fundamentals of modern genetics, this book progresses through the chemical and biological nature of molecules responsible for the storage and transfer of hereditary information, the genetic code and mutations. Molecular biology of spontaneous and induced mutations are emphasized as well as characteristics and isolation of bacterial mutants. A general discussion of recombination is followed by coverage of transformation and conjugation in bacteria. The final two chapters cover genetics of the viruses, encompassing the viruses of the bacteria, the eucaryotic microorganisms, and the higher plants and animals. *'76, 336 pp., 166 il., 35 tables, $24.75*

REGULATION OF DEPRESSED METABOLISM AND THERMOGENESIS *edited by* **L. Jansky,** *Charles Univ., Prague, Czechoslovakia, and* **X. J. Musacchia,** *Univ. of Missouri, Columbia, Missouri. Foreword by* **Charles G. Wilber.** *(30 Contributors)* Biochemistry, physiology and adaptive functions of thermoregulation are covered, combining reviews of intracellular events, electrophysiological events, and features which establish heat economy in the defense against cold. Interrelationships between the natural phenomenon of hibernation and the laboratory experimental phenomenon of hypothermia are given considerable attention. The regulatory mechanisms which take part in and occur at various levels of body temperature are also considered along with current studies of cold acclimation and new approaches to the regulation of hypothermic depressed metabolism. *'76, 304 pp., 111 il., 7 tables, $23.75*

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Fifty Years of the Ultracentrifuge

Proceedings of the Conference, Bethesda, Maryland, February 24-26, 1975

edited by MARC S. LEWIS *and* GEORGE H. WEISS, *National Institutes of Health, Bethesda, Maryland.*

Reprinted from the journal Biophysical Chemistry, Vol. 5, No. 1/2

1976 viii + 286 pages. Price: US $45.95/Dfl. 115.00
ISBN 0-7204-0511-4

An article entitled "The Ultracentrifuge, A New Instrument for the Determination of Size and Distribution of Size of Particles in Amicroscopic Colloids" was published by The Svedberg and Herman Rinde in the December 13, 1924 issue of the Journal of the American Chemical Society. Thus was described the basic instrument and techniques which J. D. Watson in "The Molecular Biology of the Gene" characterized as "Perhaps the most striking contribution of physical chemistry to the study of biological macromolecules . . .". The readers of this book should not need amplification of that statement.
In spite of the fact that these Conference Proceedings celebrate a fiftieth anniversary, ultracentrifugation is far from being a static field of scientific endeavour. For this reason, with the exception of Professor Kai Pedersen's inaugural paper reviewing the development of the ultracentrifuge in Svedberg's laboratory, the conference focussed on some representative current topics in ultracentrifugation where the major portion of the work has not been published.

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Great men and the mainstream

History of Mankind: Cultural and Scientific Development. Volume 5. Parts I–IV: The Nineteenth Century. Edited by C. Moraze. Pp. 1,394. (George Allen and Unwin: London, 1976.) Parts I–II: £15; III: £15; IV: £15.

THIS curious 'volume' appears in three substantial tomes. The various chapters in it are written by various authors, or teams of authors; many of these are based in French institutions (other European countries being little represented) while others are from the USA, the USSR, and other nations. Sometimes the author is acknowledged at the top of his contribution, whereas for others one must search among the small type at the beginning of the first tome; no credit seems to be given to the translators, who have produced smooth versions of what were presumably papers for the most part not written in English.

The work is curious for various reasons; first because it was clearly written many years ago, as indeed is admitted in the preface where a date of "before 1960" is given. We are therefore getting a warmed-up hash, many parts of which have become obsolete long since; and it is even a half-baked hash, since the bibliographies have been omitted. By this tardy and incomplete publication, justice is hardly done to the various contributors, whose reputations will not be advanced by association with this project.

Even odder is the fact that the chapters were evidently sent to referees in various countries for comments; but were then published in their original form, with the referees' comments appended. Occasionally the author has commented on these comments, and his remarks also appear. The chapters are therefore accompanied by hostile annotations, often all too justified: but because of the omission of the bibliographies, we find dogmatic statements by the author and commentator; and cannot choose between them since we do not know on what evidence they are based. To publish an historical work without a proper account of the sources used is like publishing a scientific paper without any experimental details. Even for the illustrations, we are not always told where and when they were first published, although we are told which institutions made them available.

History was the province of a muse, and one might hope that a work of history would be a work of art; but if so, one would be disappointed with this volume, in which few of the chapters are written with any verve. Some contributors have an axe to grind—this can be a great help in an historical essay—but this usually leads only to the dullness of Marxist terminlogy; whereas others are preoccupied with the hopeless attempt to leave nothing out.

It is the first tome, dealing with the history of science and technology, to which readers of *Nature* are probably most likely to turn; and we can therefore examine it in more detail. In the introduction by the editor, abstractions chase one another in what is a completely schematic and impressionistic essay, being neither narrative nor detailed case study. When we come to mathematics and astronomy, we find the account is completely 'internal' and is moreover full of sentences of the kind: "Tom, Dick, and Harry also made important contributions to this field". The Russian referee made the point that any social dimension is completely lacking; and the same can be said of the other writings on the physical sciences, those on medicine and the life sciences being better though clearly out of date.

The narratives concentrate on great men and on the mainstream, and tend to lapse into the passive voice; there is little feeling that science is carried on by people called scientists, and that an important part of its history is the study of their collective behaviour. It was in the nineteenth century that science became a profession; that science became specialised, with the general journals of the academies being supplemented by those of societies catering for one science, or part of one; that formal courses in the universities leading to first and higher degree began; and that governments and industries began to employ scientists in large numbers as new legislation and new processes were introduced. This is not apparent from these internal accounts; which is not surprising, because the emphasis in the history of science has shifted in the past twenty years or so, away from accounts of how we got to where we are now, often written by superannuated scientists, towards attempts to recover how past science actually worked and what people were trying to explain or control. These essays are monuments, of varying quality, to an elderly and amateurish tradition; the most interesting is the critical note on the history of technology by Melvin Kranzberg.

In general, therefore, it is hard to see who would ever use this monument to international cooperation, and harder to see who one would recommend to use it. **David Knight**

David Knight is a Lecturer in the History of Science at the University of Durham, UK.

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Light-emitting diodes

Light-Emitting Diodes. (Monographs in Electrical and Electronic Engineering.) By A. A. Bergh and P. J. Dean. Pp. viii+591. (Clarendon: Oxford; Oxford University: London, 1976.) £22.

THE general public first became aware of light-emitting diodes (LEDs) when pocket calculators made their dramatic entry on to the market. The miniature calculator was in fact made possible by LEDs, although the more recent development of reliable passive displays, such as liquid crystal devices, would eventually have filled the gap. For many years preceding this sudden appearance, LEDs were the subject of intense research in solid-state laboratories in many parts of the world.

The history of this period is an exciting one, with its full share of disappointments and surprises, and the authors of this book have played an important part in promoting the theory and the technology of the subject. It is disappointing that, contrary to early expectations, conversion efficiencies from electrical energy to visible light remain low in spite of the massive effort put into improving them, so the rapid technical advance of the 1960s and early 70s has now slowed down. The fact that LEDs have now found wide commercial applications, makes this a period of stability and therefore a most appropriate time for the appearance of this comprehensive work on the subject.

Nearly half of the book is devoted to a deep and detailed account of the theoretical aspects of carrier injection and the factors affecting the efficiency of light generation, most particularly in the III–V compounds. There follows a good account of the interesting field of up-converters (infrared to visible)—phosphors which seem to defy the basic rules of phosphor physics. Even though this technique has not found commercial application, it may prove of great importance if solid-state lasers are developed with output wavelengths more closely matched to the excitation band of the few available phosphors.

The remainder of the book is devoted to the technology, design and application of LEDs and associated devices, and here again the treatment is detailed and comprehensive. A minor disappointment (perhaps inevitable if the book is to be constrained to a reasonable length) is the treatment of photometry. Although a whole chapter is devoted to this subject, and many aspects are enlarged on in the chapter on LED design, the results are put in the form of generalised equations. Photometry is such a jungle of definitions, concepts and units that it is a permanent headache to the engineers trying to compare the merits of different light-emitters. An appendix containing some worked examples would have been very helpful.

The work is naturally devoted to the most theoretically tractable light-emitting devices, especially the III–V semiconductors, although there are brief discussions on SiC and ZnS. It is impressive in its deep treatment of the subject, and the enormous range of study is indicated by the collection of more than a thousand references. This makes it a valuable source-book and a necessary addition to the library of any physicist or engineer working in this field. **D. H. Mash**

D. H. Mash is a member of the staff of the Materials and Components Laboratory, Standard Telecommunication Laboratories Limited, Harlow, Essex, UK.

Reptilian physiology

Biology of the Reptilia. Vol. 5: Physiology. Edited by C. C. Gans and T. S. Dawson. Pp. xv+556. (Academic: London and New York, October 1976.) £16.80.

THE preceding volumes of this encyclopaedic survey, which appeared between 1969 and 1973, were devoted to morphology; and herpetologists interested in physiology have had to await patiently the turn of their own speciality. The first of the physiological series, however, should not disappoint them. The literature on the subject is remarkably scattered because reptiles have been studied, not only in their own right but also as subjects for physiological and pharmacological assay. In the book under review, an attempt has been made to provide an overall summary orientated towards reptiles as a taxon rather than towards single topics such as metabolism, thermoregulation, or water balance. In this endeavour, the editors have enlisted authors who, in each case, have contributed signally towards elucidating the particular topics they summarise. The cast is all-American, but at least the book was printed and bound in Great Britain.

In their opening chapter, entitled 'Reptilian physiology: an overview', the editors emphasise the ecological aspects of environmental physiology. This is followed by a detailed discussion of 'Methods for the physiological study of reptiles' by Harry S. McDonald. Subsequent chapters are as follows: 'Metabolism' by Albert F. Bennett and William R. Dawson; 'Respiration: mechanics, control and gas exchange' by Stephen C. Wood and Claude J. M. Lenfant; 'Circulation' by Fred N. White; 'Regulation of acid-base balance in reptiles' by Barbara J. Howell and Hermann Rahn; 'Osmo-regulation' by P. J. Bentley; 'Salt glands in reptiles' by William A. Dunson; and 'Renal function (with special emphasis on nitrogen excretion)' by William H. Dantzler. The volume concludes with a comprehensive index, but this does not seem to have been checked with quite the thoroughness usually characteristic of Academic Press.

Throughout, the reader will be struck by the heterogeneity of the Reptilia, which is reflected in their physiology. For instance, in many reptiles the salt gland is the major route for excretion of electrolytes; yet these glands have evolved independently, over and over again, in the process of adjustment to dry or saline environments. Great variation exists in the regulation of nitrogenous excretion, and this too may be related to habitat as well as to the end products of metabolism, the ability of the cloaca or bladder to regulate the volume and composition of the urine, and the ability to excrete ions by extra-renal routes. Similarly, metabolic rates are extremely variable, but little attention has yet been directed towards their functional correlates. The vast diversity of respiratory mechanics, lung morphology and gas transport mechanisms, is matched by comparable diversity in habitats, habits and levels of activity. The heterogeneity referred to above is thus clearly related not only to phylogenetic divergence but also to adaptive radiation within the various orders and suborders.

The search among living reptiles for intermediate evolutionary linkages with mammals and birds has diverted attention from the adaptive features and control mechanisms of the reptilian cardiovascular system. Yet, such features and mechanisms form an important part of the integrative physiology of the class. Reptiles have, potentially, a large contribution to make both to cellular biology and to ecology, and the future of reptilian comparative physiology should be linked closely with these areas.

Relatively few of the physiological processes discussed in this important volume have yet been investigated intensively, and any such studies comprise only a few species. Much work remains to be carried out before reptilian physiology can be understood really adequately. This book plays an important rôle in emphasising both what is, and what is not, now known.

J. L. Cloudsley-Thompson

J. L. Cloudsley-Thompson is Professor of Zoology at Birkbeck College, University of London, UK.

Molluscan delights

Pseudothecosemata, Gymnosomata and Heteropoda (Gastropoda). By S. Van Der Spoel. Pp. 484. (Bohn, Scheltema and Holkema: Utrecht, 1976.)

As once aptly observed by Dr Joel Hedgpeth, taxonomy is man's oldest profession, his "first task in the Garden was not to work for his living—that came after the Fall—but to name every beast of the field and every fowl of the air". Too soon interrupted in this, it has been left to succeeding generations to complete a task which increased in size with every new exploration, including the waters of the sea, and with every development in the capacity for magnification.

The significance of taxonomy was enhanced with the demonstration of the fact of evolution. What had been accepted as the works of a highly ingenious, if in certain aspects a by no means beneficent, Creator now appeared as the contemporary endpoints of innumerable, often interlocking, lines of evolutionary change. Taxonomy records the outcome of the most significant sequences of events that have occurred during the history of this planet.

To a marine malacologist it is difficult to imagine two more fascinating groups than prosobranch Heteropoda and opisthobranch Pteropoda (Euthecosomata, Pseudothecosomata and Gymnosomata) evolving independently as holoplanktonic gastropods. It is a delight to see with what elegance they are treated in this volume by Dr S. Van Der Spoel of the Institute of Taxonomic Zoology, Amsterdam. It succeeds an earlier one, in 1967, dealing solely with the Euthecosomata and is to be followed by a third concerned with the biology of all planktonic molluscs.

This surely is an ideal example of the manner in which the products of evolution should be treated with adequate description and synonomy of every species, accompanied by 166 beautifully drawn line figures—and how fully these exquisite animals merit such attention—followed by 81 maps showing world distribution. Appendices contain diagrams revealing the diversity of taxa in the different faunal centres with others assisting in the use of keys for identifying species difficult to separate, notably in the heteropod Atlantidae. Recent information about the Euthecosomata brings the earlier volume up-to-date, and a reference list of over 80 pages covers the entire literature.

Differing widely in origin, the heteropods and pteropods differ also in distribution, a possible consequence of their different periods of origin. There are more heteropods in the Indo-Pacific but pteropods are commoner in the Atlantic. From this it is postulated that the former evolved in the greater oceanic area. But, being more thermophilous, they found difficulty in passing through colder southern waters into the Atlantic. They are not, however, absent in that ocean and nowhere do more spectacular species of both groups live together than in the Mediterranean, where this reviewer has found much pleasure in studying living representatives.

Dr Van Der Spoel's book is a work of combined scholarship and research and forms a permanent addition of the highest value to zoological knowledge. It should be contained in every museum, every marine laboratory and every University library. It is an indispensible work of reference to marine biologists, both applied and academic. It will be a delight to every malacologist.

C. M. Yonge

Sir Maurice Yonge, formerly Professor of Zoology at Bristol, then at Glasgow, is Honorary Fellow in the University of Edinburgh, UK.

Biology of Kinetoplastid flagellates

Biology of the Kinetoplastida. Vol. 1. Edited by W. H. R. Lumsden and D. A. Evans. Pp. xxvi+563. (Academic: London, New York and San Francisco, 1976.) £18.00.

The parasitic members of the flagellate order Kinetoplastida of the phylum Protozoa have always attracted more attention than the free-living forms, and the species of two genera, *Trypanosoma* and *Leishmania*, which infect man, have attracted the most. It is easy to forget that these genera are only two out of sixteen or more, that many trypanosomes cause no harm to their hosts and that it might be possible to learn as much about trypanosomiasis and leishmaniasis from comparative studies on related forms as from the trypanosomes and leishmanias of man.

To many protozoologists, the literature is top-heavy with information on the Kinetoplastid flagellates of medical and veterinary importance and it is the intention of this multi-author volume (and its companion when it is published) to redress this imbalance and to present the Kinetoplastida as a whole. Difficult as this is to achieve, it looks as if the two volumes between them will do just that.

This volume, for a number of reasons explained by the editors, is mainly concerned with trypanosomes, apart from the first two chapters (by K. Vickerman, and K. Vickerman and T. M. Preston) which deal with the diversity and cell biology of the kinetoplastid flagellates as a whole. Both these chapters contain a considerable amount of original thought and research material. There are also four comprehensive reviews of trypanosomes, of birds (J. R. Baker), of bats (C. J. Marinkelle), of primates other than man (C. J. Marinkelle) and of the subgenus *Herpetosoma* (D. H. Molyneux). These, with their well presented tables and check lists, will be invaluable sources of information for years to come.

Two of the remaining chapters cover the rapidly advancing fields of the biochemical aspects of taxonomy (B. A. Newton) and oxidative metabolism (I. B. R. Bowman and I. W. Flynn). Informative and useful as these are at present they will be even more useful when read in conjunction with the chapters on the taxonomy of the leishmanias and biochemical aspects of *Trypanosoma cruzi* which are promised in volume 2.

Two further chapters deal with the subgenus *Megatrypanum* (E. A. Wells) and *Trypanosoma rangeli* (A. D'Alessandro). Although their inclusion can be justified on the grounds that the information contained in them is not adequately summarised elsewhere, they are really too specialised to justify such extensive coverage in a book of this kind. The remaining two chapters on innate resistance to trypanosome infection (R. J. Terry) and antigenic variation (A. R. Gray and A. G. Luckins) are again rather specialised, and would have been better associated with other papers on immunity also due to be published in volume 2. In any case, antigenic variation could do with a rest from reviews for a year or so.

This is a most valuable work of reference but could have been so much better if the reproduction of the plates and electron micrographs had been of better quality, and if all the chapters had been updated from their original 1973 form as some of them have been. The publication of volume 2 is eagerly awaited. Perhaps it would not be too much to hope that each chapter in volume 1 will be briefly updated and that better quality paper will be used for the plates.

F. E. G. Cox

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Protein crystallography

Protein Crystallography. By T. L. Blundell and L. N. Johnson. Pp. xiv+565. (Academic: London, New York and San Francisco, 1976.) £17.00.

FOURTEEN years after the award of Nobel prizes to Crick, Kendrew and Perutz, this is the first textbook to be published on protein crystallography by an established practitioner in the field. Perhaps this indicates the compulsive preoccupation of the protein crystallographer with the mechanics of his work, but in any case the event is to be welcomed.

One of the attractions of protein crystallography is that narrow specialisations are not allowed. Without sound grounding in mathematics, physics, crystallography, inorganic chemistry, organic chemistry, biochemistry and biology, one will constantly run into problems one cannot evaluate. The experimenter needs in addition a smattering of knowledge of instrument design, high vacuum technology, electronics, chemical engineering and computer science. Such versatility is demonstrated admirably by the authors.

The character of the book as a work of scholarship first emerges in a discussion of the selection of heavy metal compounds for introduction into proteins to provide isomorphous crystalline derivatives. The chemistry of different classes of transition metals and the properties of various categories of complex ion are related to the types of isomorphous substitution which have been obtained. Much of what is offered has not appeared in any review—it is an original arrangement and interpretation of the evidence leading to conclusions which are perfectly orthodox as inorganic chemistry, but which will profoundly change the strategy which protein crystallographers adopt.

Another penetrating analysis deals with an unresolved conflict in the literature between two methods for the exploitation of anomalous scattering effects, due to A. C. T. North and B. W. Matthews. The germ of the idea is traced back to an Oxford thesis by M. Harding; we are led forward to an analysis with no approximations by Singh and Ramaseshan; a tacit assumption in Matthews' choice of the positive value of a square root is pointed out, and his method is clearly related to the "FHLE" method.

In a survey which ranges from film developing technique and microdensitometry to the exploitation of electron diffraction and Mössbauer scattering there are many other stimulating and original insights. The expert will derive refreshment and pleasure from them. Unfortunately the beginner will be less satisfied. The authors have provided introductory chapters which seem to be aimed at students who know no biochemistry and no crystallography. These chapters make no concessions to the problems of teaching these subjects, and provide an inappropriate *hors d'oeuvre* to the main meal. Some of the obscurities are simply slipshod (that hydrolysis of proteins with boiling mineral acids yields mixtures of up to twenty amino acids), but others will cause serious confusion (the only definition of a Fourier transform is to use it as a synonym for diffraction pattern, for example).

In practice the price of the volume will keep it out of the hands of students, at least in Europe, and it seems a pity that the authors should have misdirected so much effort to cater for them. A good student text is still desperately needed. The book is splendidly up-to-date, including many 1976 references. For some reason the first few pages look rather as though they have been set on a pre-War typewriter, and occasionally one can see evidence of proof corrections, but otherwise the book is well produced. It was a surprise to see several familiar illustrations without acknowledgment.

David Blow

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Plutonium and other actinides

Transuranium Nuclides in the Environment. (Proceedings of a Symposium organised by the US ERDA and the IAEA, San Francisco, 1975.) Pp. 724. (IAEA: Vienna; HMSO: London, 1976.) £22.88.

THESE 41 technical papers form an indispensable basis for assessing the "growing concern about man's ignorance of the biogeochemistry of [plutonium and other actinides] . . . in relation to . . . long-term buildup, availability, and transport in the environment" (Noshkin). Nowhere is this concern more apt than the UK, which reportedly discharges to the Irish Sea some 0.3% of its Pu throughput—of the order of 10^4 Ci since 1960.

The message of these papers is clear: our ignorance of pathways is extensive for Pu and is virtually complete for the higher actinides (such as Am) that are more active biochemically and that may be the dominant hazard. Among the wide range of concentration factors for Pu and higher actinides—from none to several orders of magnitude per trophic level, and from strong discrimination to modest concentration in food chains—exceptions are more common than rules. Unexpected interactions with soil microbes, natural chelates, marine worms, mammalian digestive fluids, and fungi (some of which incorporate actinides into aerial spores) likewise falsify familiar axioms. Particle size is important to these effects, to astonishingly high mobility in soil, to resuspension and adsorption on plant leaves, and to human inhalation (generally a greater hazard than ingestion). Even the chemical behaviour of, say, ^{238}Pu and ^{239}Pu differ markedly. And Pu contamination is indeed ubiquitous: quoted ranges exceed 40 km for routine emissions of a US reprocessing plant, 80 vertical m for bomb residues in coral, and 500 km for dust from Nevada dispersion tests. Both on land and in water, Pu concentrations can be far lower *at* a point source than at distant sites of favoured deposition.

The most impressive and disquieting example cited is G. L. Meyer's celebrated account of Maxey Flats, Kentucky, a site containing some two-thirds of all US commercial low level nuclear wastes buried during 1963–74. Over 1.6 MCi, including over 80 kg ^{239}Pu and unknown amounts of 300-odd other nuclides, were buried in steel drums and in wood and cardboard boxes. The trenches, in a humid forest underlain by jointed and leaky strata, promptly flooded. Runoff, groundwater, and wind carried contamination off-site (hundreds of metres in a decade) by every 'normal' route plus a few new ones, raising "serious questions about knowledge and concepts of plutonium mobility in the environment"—to say nothing of management. But a British participant, rightly questioning the suitability of the site, was told it seemed preferable to marine discharge, and the marine pathway analyses in other papers hardly contradict this.

The book's attempt to deal not only with actinide pathways but also with the biomedical effects of exposure suffers from too little space and diversity. Although current standards, linearity, neglect of isotopic chemical differences and of gonadal dose, and conventional dosimetry are all properly queried, the brief and truncated reviews hardly do justice to the richness of the controversy. In summary, an important reference work with strong relevance to public policy.

Amory B. Lovins

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nature *February 3, 1977*

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That Was the Weak Force, That Was

For all that science is the great emergent culture of the twentieth century and that its ground rules are known to more educated men and women than those of any other cultural activity, it is still a strangely back-room affair as far as most members of the general public are concerned. There is more than residual belief that scientists should stick to science and that non-scientists can't be expected to understand a word of it. For this reason we have a lot to be grateful for in the offerings of BBC Television's *Horizon* as a long-running and generally successful attempt to portray a vast and comprehensible world of science in the terms of its human practitioners. We also have reason to appreciate the occasional televised *Controversy* debate as a sign that the scientist can have as important things to tell the public as do politicians, economists and administrators. But should we feel the same way about the occasional 2-hour-or-more programmes that BBC screens under Mr Nigel Calder's guidance? The double showing in Britain this week of *The Key to the Universe* has raised again nagging doubts that have surrounded Mr Calder's previous presentations: *The Violent Universe, The Mind of Man, The Restless Earth, The Life Game, The Weather Machine* and *The Human Conspiracy*.

It is Mr Calder's belief, put forward in *Nature* **245,** 293 (1973), that close to the frontiers, communication of science to the layman is easier than in well-established areas because of the excitement, the starkness of the questions, the lack of jargon. Unfortunately, it is not equally evident that two hours is the right length of time to be spent on the subject, or that everything exciting within the subject should be covered. In the above-mentioned article Mr Calder himself described the problems of an overabundance of material, but he has often created the impression of not having pruned hard enough; the result has been a bewildering patchwork the underlying unity of which is not at all clear. In this most recent programme he managed to mention (in this order) neutrinos; the four forces; quantum mechanics; Feynmann diagrams; gravitons; gauge theory; Weinberg-Salam theory; renormalisation; 'heavy electrons' (muons); neutral currents; strangeness; the Ω-particle; quarks; colour; gluons; strings of gluons; anti-matter; exploding galaxies; radio background; big bang; lost symmetries; gravity 'frozen out'; latent symmetry . . . before the interval. We saw at least seventeen high-energy or astronomical facilities. We were introduced colloquially to 'cosmic alchemy', 'pattern makers', the 'starbreaker', the 'fire-maker' force, our 'phantom friends', 'old man gravity', 'crazy ideas', 'spooky' black holes. We were treated to verbal infelicities like 'they are no luxuries, the electrical and mechanical aids at the Inter-Universities Institute for High Energies', and visual infelicities like the bizarre rope bridge from which Mr Calder declaimed, at times Messiah-like.

The initiated found a certain amount to admire in all this—new insights, nice turns of phrase, some clever visual work. The uninitiated, which must have been 99.9% of the audience, can only have been thoroughly confused by the profusion of jewels in the crown which were picked up in no apparent order, admired and put back, and with few concessions to those who blinked at the wrong moment.

Mr Calder and his team make one fundamental mistake: they underestimate the serious-mindedness of the audience. Viewers are not there by mistake or because they have been watching the previous programme; they are prepared to settle down for the required couple of hours to learn and many are even prepared to watch the programme twice. Such viewers don't need a breathless approach, they are prepared to devote time to fixing new ideas in their mind, if necessary by repetition, by schoolroom techniques and by relation to everyday experiences. They would also appreciate an historical account of why we are where we are; twenty minutes or half-an-hour of bringing the average viewer up to speed would indeed erode the number of exciting new concepts that could be crammed in, but it might ensure that the understanding of those concepts retained was more profound.

There is a serious danger in the presentation of science to the public that it should seem too esoteric and confusing. This widens the rift between scientists and non-scientists and does little to help persuade the young to turn to science. Mr Calder has consistently tried to avoid this by getting right amongst the discoveries as they come out, and in principle he is correct. But in practice the erudition he acquires in researching his programmes ends up overloading his audience, who may come to just the wrong conclusion that science indeed is somewhat too esoteric and rather too confusing for them. □

What role for fission?

Leslie Shepherd assesses the contribution nuclear fission can make to the energy requirements of the next 50 years

DURING the 1960s annual world energy demand was expanding at a rate of 4.5% a year. If that trend had continued, then 50 years from now the energy demand would be almost ten times as great as at present. With the emphasis that is now being set on the need for more careful and efficient use of energy, however, a lower rate of growth of perhaps 3.5% a year seems more probable for the future.

On this basis, 50 years from now we should be consuming primary energy at a rate of 1.5×10^{18} kilojoules a year and the cumulative consumption in those 50 years would be 3×10^{19} kilojoules. It is extremely unlikely that more than two-thirds of this could be met by fossil fuels because of the limitation on the total economic resources of petroleum oil and natural gas ($\sim10^{19}$ kilojoules) and the feasible rate of extraction in the case of solid fuels. In fact, if the mining of coal and lignite were steadily increased by a factor of 5 over the next 50 years, the cumulative energy yield from these would be no more than 10^{19} kilojoules.

Nuclear fission, as the only practical alternative energy source to coal and oil on the scale which we are concerned with, therefore, may have to contribute at least one-third of the cumulative consumption by the year 2025 and thereafter provide the bulk of the future demand.

Uranium utilisation

Since uranium is the only natural source of fissile material, U-235, it is the basic raw material of nuclear power. One tonne of natural uranium contains sufficient U-235 to yield 5×10^{11} kilojoules of energy. The OECD's survey listing the reasonably assured and estimated additional resources of uranium from rich ores, published in December 1975, gives a current total of 3.5 million tonnes, the U-235 content of which, on its own, would yield only 1.75×10^{18} kilojoules. This is not a very impressive amount of energy—no more, in fact, than the present measured reserves of natural gas would yield.

Fortunately, the real reserves of fission energy, if they are properly exploited, are certain to be orders of magnitude greater than this figure suggests. This is partly because exploration of uranium resources is still in its early stages and the actual amounts economically extractable are certain to be far greater than indicated in contemporary estimates. In addition, nuclear reactors not only consume the fissile U-235 charged into them, but also generate fresh fissile material by neutron absorption in the so-called fertile isotopes U-238 (which constitutes more than 99% of natural uranium) and Th-232 (the whole of natural thorium); this process of converting fertile to fissile material enables us to increase the fissionable content of nuclear fuel many fold.

The principal fissile isotopes resulting from these fertile captures are Pu-239 (from U-238) and U-233 (from Th-232). The amount of energy which we are able to derive from the originally mined natural uranium (and thorium) will be determined by the extent to which these secondary fissile isotopes can be generated and utilised starting from the primary U-235. This is a question of the 'neutron economies' of the various possible reactor systems and the fuel cycles adopted in them—the Uranium Cycle involving U-238/Pu-239, and the Thorium Cycle involving Th-232/U-233.

Not all of the neutrons absorbed in U-233, U-235 and Pu-239 cause fission; a proportion of the neutrons captured produce the higher isotopes U-234, U-236 and Pu-240, which in turn can absorb further neutrons yielding still higher isotopes. In fuels which have been in a reactor for some time the fissile components build up into a chain of isotopes (U-233, U-234, U-235, U-236 in the thorium fuel cycle, and Pu-239, Pu-240, Pu-241, Pu-242 in the uranium cycle). All of these isotopes have to be taken into account in evaluating neutron economy.

Neutron economy

Neutron economy refers to the balance of the processes which produce and absorb neutrons in a nuclear reactor. Each fission is the result of the absorption of a neutron in a fissile nucleus, and in the process fresh neutrons are emitted. One of these is required to produce a further fission, the balance going either into productive or non-productive processes. The productive process is neutron-absorption in the fertile isotopes, U-238 or Th-232, which yields new fissile material. Non-productive processes include:

- neutron-capture in the fixed materials making up the reactor core;
- absorption in fission products as they build up and in the control rods which are introduced to maintain the neutron balance as the fuel burns up;
- the non-fission neutron capture in the fissile species, which was mentioned above (the ratio of non-fission captures to fission captures is referred to as the α-value of the fissile species in question);
- neutron leakage from the reactor core.

It is, of course, the neutron absorption in fertile material, leading to the generation of fresh fissile isotopes, which is important, since it determines the ultimate amount of energy which may be derived from uranium and thorium resources. The ratio of fresh fissile atoms produced to those consumed in the chain of events described is known as the conversion ratio. If this is less than unity the amount of fissile material in the reactor steadily diminishes during operation and loss has to be made up periodically. However, if the ratio is greater than unity (it is then called the breeding ratio), there is a surplus of fissile materials available for other reactors and the system is said to breed.

Neutron economy varies according to the type of reactor and the manner in which it is operated; it also depends on the fuel cycle (uranium or thorium) adopted. The optimum situation is achievable in fast reactors, where breeding is possible largely because of the relatively low α-values in all the fissile materials when the neutron energy is high.

The uranium cycle is better than the thorium cycle in fast systems. This is because of the greater yield of neutrons in the fission of Pu-239 as compared with U-233, and because the most energetic neutrons can cause direct fission of the fertile U-238 (an effect which is far less pronounced in Th-232) and, by this means, boost significantly the number of available neutrons. In thermal reactors, the α-value is very high for Pu-239 (~0.6) and low in the case of U-233 (~0.1) with the result that much higher conversion ratios are possible with a thorium fuel cycle than with a uranium fuel cycle.

Thus from the standpoint of achieving the maximum energy output from natural uranium resources there is an incentive to develop fast reactors with the uranium cycle and to use the thorium fuel cycle in thermal reactors.

Thermal reactor characteristics

In a global context three categories of thermal reactor are of interest: light water reactors (LWR), heavy water reactors (HWR) and the helium-cooled

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high temperature reactor (HTR).

As far as neutron loss in the moderator is concerned, light water is worst, carbon is intermediate, while heavy water is essentially non-absorbing. The same remarks apply to light and heavy water as coolants; no neutrons would be absorbed in a helium coolant. LWR and HWR cores both incorporate a large amount of neutron absorbing metal in fuel element cladding and pressure tubes, so that these systems are appreciably worse in this respect than the HTR which includes no metal structure. Taking these factors into account both the HTR and the HWR have an advantage over the LWR.

The proportion of neutrons lost by absorption in fission products (more or less independent of reactor type) depends upon the extent to which these are allowed to build up relative to the core inventory of fissile material, that is to say the degree of fuel burn-up before it is discharged from the reactor for reprocessing. Reprocessing separates out the fission products from the unburned fissile material which can then be recycled in the reactor. Reprocessing is an expensive matter and for that reason among others it is desirable to keep a high inventory of fissile material in the reactor core (if a good neutron economy is desired) to avoid too frequent recycling.

The yardstick in the evaluation of future thermal reactors is the current uranium fuel cycle LWRs. These operate at present with no recycle. The conversion ratio in the single fuel cycle is about 0.60, and more than half of the Pu-239 produced is actually consumed in this cycle. An LWR of 1000 MW gross capacity, operated in this way for 30 years at 80% load factor, would require 6,000 tonnes of natural uranium. At this rate about 25 million tonnes of uranium would be required to yield the 10^{19} kilojoules indicated above as a cumulative energy target over the next 50 years. This amount could be reduced by about 40% if fuel were recycled to use up all residual U-235 and plutonium.

Both the HWR and the HTR would achieve a better performance than the LWR with the uranium fuel cycle for the reasons already given. In the case of the HTR, the superior thermodynamic efficiency of the power conversion cycle*, arising from the higher temperatures achieved, would also mean that the useful energy ultimately derived would be substantially higher than with the water cooled reactors.

The biggest gain, however, would come from the use of the thorium fuel cycle in thermal reactors because of the greater yield of neutrons from U-233 compared with Pu-239. This would carry a potential advantage of about 0.4 in conversion ratio compared with the uranium cycle LWR and even introduce the possibility of operating thermal reactors as breeders. However, this would require very large inventories of fissile material in these reactors so that the initial large requirement of mined uranium would offset the advantage of the breeding for a very long period, certainly longer than the 50 years considered here.

Furthermore, U-235 being the only naturally occurring fissile material, all nuclear fuel cycles must begin with this isotope. This means that there is an initial phase in which the neutron economy is determined partially by the characteristics of U-235, with a consequently lower conversion ratio during that phase.

High conversion thorium fuel cycle thermal reactors would require some 5–7 million tonnes of uranium to be mined over the next 50 years in order to achieve an energy output of 10^{19} kilojoules. This is in excess of the presently estimated resources of uranium from high grade ores. Without doubt, the present figures greatly underestimate the actual available resources, particularly if one takes into account the acceptability of using much more costly uranium in high conversion or breeding systems.

At the end of that time, however, at the 3.5%-a-year rate of energy growth the annual nuclear energy demand would be $\sim 10^{18}$ kilojoules and would be increasing. It is difficult to envisage meeting this requirement without having built up an adequate breeder reactor capacity. This provides the incentive to develop a technically and economically viable fast reactor and to have such a system established on a substantial scale 50 years from now.

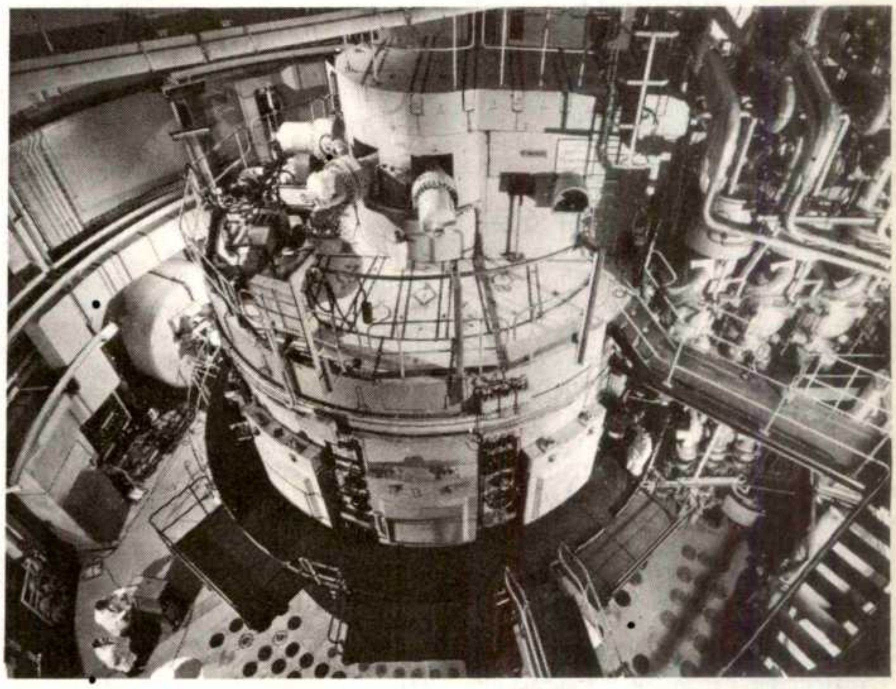
Fast reactor exploitation

A fast reactor using plutonium will have an initial induction phase in going from U-235 to Pu-239, as would be the case in building up a U-233 inventory in a thermal reactor. Currently, the accepted strategy is to use thermal reactors (predominantly LWRs) to produce the necessary plutonium inventories for fast breeders. Unfortunately, this is a particularly inefficient process, a non-recycle LWR yielding only about a quarter of a kilogramme of fissile plutonium per MW-year.

Taking four tonnes as being a minimum plutonium inventory for a sodium-cooled fast breeder of 1000 MW capacity (2.5 tonnes in the core and 1.5 tonnes in recycle), its production would require an LWR of corresponding power output to operate for 20 years at 80% load factor, consuming more than 3,000 tonnes of uranium in the process.

Subsequently, the fast reactor would yield excess plutonium which would be available to build up further inventories. There is a possibility, however, that the doubling time—that is to say the time for the fast reactor to generate sufficient excess plutonium to provide a further reactor inventory—may be very long, which would make for an excessively slow build-up in the ratio of fast reactors to LWRs.

In such circumstances there would be, certainly over the next 50 years, an unacceptably high preponderance of



UKAEA

A view of the Dragon

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*An electricity generating HWR would have a thermal efficiency of 30% compared with 40% for a steam cycle HTR and 45–50% for a binary cycle HTR.

LWRs over FBRs, so that one would be far removed from an overall breeding situation and the total uranium demand could be significantly higher than for a programme based only upon high-conversion thorium fuel cycle reactors.

This approach is unnecessary, however, it being much more logical to start fast reactors with U-235 and produce the eventual Pu-239 inventory *in situ*. In this way all of the plutonium would be produced and consumed in its most favourable environment. The time to produce a full plutonium inventory and, therefore, a true breeding situation would be much shorter and the uranium input very much reduced, possibly by a factor of four, compared with the LWR approach.

A further advantage of this course is that it would make the fast reactor independent of any thermal system, and it would relieve the latter of the requirement to work on the uranium fuel cycle in order to provide plutonium for fast systems.

While thermal reactors are not appropriate sources of plutonium for fast reactors, it is worth stressing that the converse is not true, insofar as U-233 could be generated efficiently in thorium introduced into the blanket of a fast breeder reactor. In this way a fast reactor with a good breeding ratio could support high conversion thermal reactors with a combined energy output several times greater than its own.

This is an important possibility leading to a symbiosis of a small number of fast breeders with a larger number of high conversion helium-cooled high temperature reactors, which would be preferred to the fast reactor on all counts other than neutron economy.

Conclusion

In the foregoing a world viewpoint has been taken deliberately, because no individual nation can hope to proceed in an isolated manner in resolving problems of such significance as those presented by the energy situation.

Unless some viable major alternative energy source (such as nuclear fusion) comes along, there will be a minimum cumulative requirement of 10^{19} kilojoules of thermal energy from nuclear fission over the next 50 years, and at the end of that time the annual energy contribution will need to be 10^{18} kilojoules a year. The requirement is far beyond the capabilities of light water reactors working on their present uranium fuel cycles, but it would not be beyond the capacity of thorium fuel cycle thermal reactors, notably the high temperature reactor, provided that they were being followed up with a well established programme of fast reactors.

However, the strategy of using thermal reactors (predominantly LWRs) to produce the plutonium inventories needed for breeding does not appear to be viable. The rapid exploitation of the breeding capabilities of fast reactors requires that they should generate their own plutonium from the initially available U-235. On this basis the evolution of fast reactor exploitation would proceed independently of the programme of thermal reactors. Eventually, the two would come together in a symbiosis, in which an overall breeding situation would be maintained with a minimum of fast reactors. ☐

FRANCE

Debating nuclear power

Alexander Dorozynski reports from France on the nuclear power debate there

THIS year may be decisive for the ambitious French programme of rapid development of nuclear energy. Well before the debate triggered by the "all nuclear" decision of the Pierre Messmer government in March 1974 physicists and ecologists started voicing worries about the programme's safety and reliability, and the doubters appear still to be growing in numbers and in political weight.

The controversy has spread beyond the potential dangers of a technology that has not been entirely mastered. Those arguments find a focus in the huge nuclear waste treatment plant in La Hague, at the tip of the Cotentin directly south of Bournemouth, and in the Superphénix fast breeder reactor, to be located just 44 km from Lyon. The debate now extends to many of the economic and political arguments advanced by the powerful Electricité de France (EDF), the state monopoly employing some 100,000 persons and one of France's largest and most powerful enterprises.

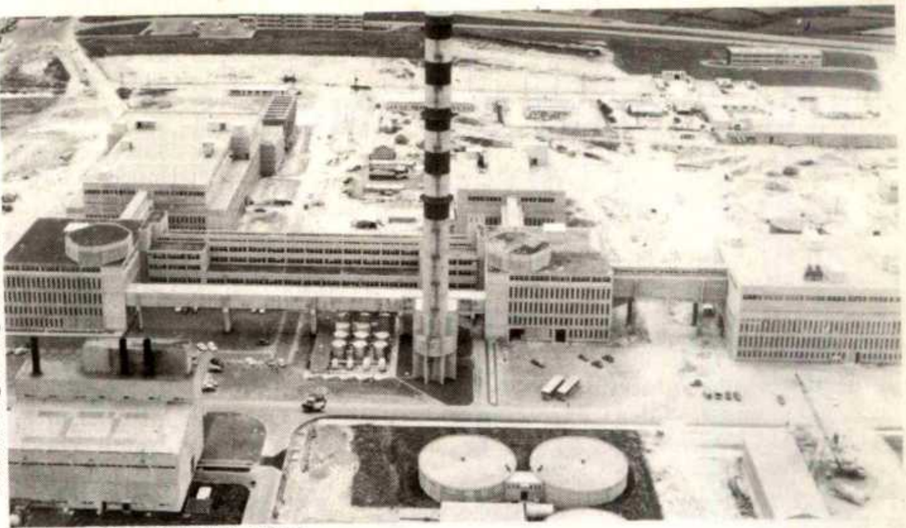
The Ministry of Industry and Research has been only marginally effective in dealing with the 'dissidents', and in the face of mounting public awareness, a public relation campaign is being mounted by EDF. Embarrassingly, some of the approaches to be taken have been revealed by an ecological weekly, *La Gueule Ouverte*, which has published a confidential EDF document giving recommendations about how to deal with different categories of opponents, such as the public at large, members of the parliament, physicians, teachers, journalists, and so on.

Now another publication, *Science et Vie*, France's largest science magazine, is publishing the results of a debate it has organised about the economic soundness of the programme. The debate put face to face economists from the EDF and members of the Institut Economique et Juridique de l'Energie (IEJE), a small group formed at the University of Social Sciences of Grenoble. A guest participant was Professor Irvin C. Bupp, Jr, a Harvard University economist who has taken part in a MIT study of the evolution of the price of water reactors in the US—PWRs are being installed in France under a patent agreement with Westinghouse.

The initial nuclear programme has already been toned down under Valery Giscard d'Estaing's presidency: annual plant construction will be decreased from a 6,000 MW capacity in 1976 and 1977 to 5,000 MW in 1978, and the goal of producing by 1985 nuclear energy equivalent to 60 million tons of oil has been adjusted downward to 55 million. But until now, few had challenged the figures authoritatively produced by EDF concerning the price of the nuclear kilowatt, and the cost of nuclear plants.

"The only economists who have ventured to challenge official figures are those of the IEJE," points out one participant, Jean Marie Chevalier, professor of economics at the University of Paris-Dauphine. "In fact, the nuclear programme has been imposed upon the country by EDF and the Commissariat a l'Energie Atomique (CEA). Never has there been the slightest counter-expertise. The government let it go without compromising itself. Thus, if need be, it will be able to reject upon EDF the responsibility of the aberrant situation we might end up with." The figures are now likely to be subjected to close scrutiny.

For instance, EDF economists have tagged the nuclear KWh with a cost of 7.5 centimes, while that of the 'classical', oil-fired KWh varies between 11.5 and 12.5 centimes. IEJE economists ask if this price is realistic. Just across the border, in West Germany, the nuclear KWh is priced at 6 pfennig (12 centimes), and Patrice Romain of the IEJE maintains



Reprocessing plant at La Hague

Nuclear Engineering International

that if engineering costs, insurance, cost of processing waste and of dismantling reactors are included, the EDF price goes up steeply. Then there is the cost of uranium, which has gone up from \$5 a pound in 1973 to \$40 a pound. Nuclear fuel cost, once estimated at 20% of total costs, has now reached 30% and is still growing. If waste treatment is included, notes Jean-Marie Chevalier, it may reach 60%.

Similarly, the cost of constructing nuclear power plants is challenged. Is it closer to the FF2,150 per installed KWh, as quoted by EDF, or to FF6,700, the price France would like to charge for similar installations in Iran? Does this difference mean that a cost in constant francs must be upped by 50–100% for a foreign country, that more safety factors are included for export, or that EDF discounts some hidden costs that appear elsewhere in its budget? In the United States, Bupp notes, this cost was \$130 per installed KWh in 1965, \$300 in 1972, and is estimated today at some \$700 (about FF3,500).

Another major argument frequently advanced for the intensive development of nuclear power is also being challenged: that of energy independence. According to a report to the National Assembly, French requirements in uranium from now to 1985 are estimated at 85,000 tonnes, and by 1990 at between 115,000 and 125,000 tonnes. "If France had to count on its own resources, these would be exhausted between these two dates," states the report. France may have interests in mining operations in Niger, Gabon and Canada, but it is wondered if there is a guarantee that these interests will be preserved.

There are questions, too, about France's real independence with regards to enrichment. France's uranium enrichment plant at Pierrelatte, designed for military purposes, has insufficient production capacities. The Tricastin plant nearby should only be operative in 1980—if all goes well.

None of the 'dissidents', whether physicists or economists, argue against nuclear power as such. Their major objection is that the EDF plans for producing energy from the atom and using electricity as a vector have closed the door to alternatives. In 1977, only FF30 million will be devoted to research in new energy sources, as against FF2,474 million budgeted for CEA projects.

"We are making a mistake by not playing the card of natural gas," adds Chevalier. "Gas is found everywhere—in North Africa, the Middle East, the Soviet Union, Holland, under the North Sea. It is energy which, unlike oil, is not dominated by multinational firms. . . Contracts between state and state could be made to preserve equitably the interests of both parties. And gas is a decentralising energy, unlike nuclear power, which leads to the development of maxi-sites and of national distribution networks. With gas turbines, we can produce locally and on a small scale electricity and heat, thereby reducing social costs."

Chevalier believes that no other form of energy can be made available as rapidly as gas, and that within four years, a flow could be created that would represent a saving of FF25–35,000 million. Meanwhile France, which imports three-quarters of the energy she consumes, could explore alternatives before the nuclear 'solution' becomes irreversible. ☐

● **Azim Kidwai writes from Karachi:** Although nuclear cooperation between Canada and Pakistan has ended (see *Nature*, January 6, page 9, and also January 13, page 95), the deal with France for the supply of a nuclear reprocessing plant still stands. The plant was one of the main bones of contention in the dispute with Canada. Considerable pressure on Pakistan to cancel the French deal also came from the USA. Pakistan defended the right of two sovereign states to enter into such agreements, but to allay the fears of those who thought that the plant would be used for other than peaceful purposes, it showed its willingness to negotiate further safeguards even though the agreement had been cleared by the International Atomic Energy Agency.

The ending of Canadian assistance will have its effects. The only nuclear power plant running in Pakistan, the 137 MW Karachi nuclear power plant (KANUPP), was supplied by Canada. Its fuel, essential spare parts and the technical assistance for its maintenance were from Canada. A policy of self-reliance may now be the order of the day, and the Chinese experience when Russian technical assistance was withdrawn in the early 1960s is being cited.

The supply of fuel to KANUPP may pose no problems, as fuel fabrication is likely to be arranged from sources other than Canada. Eventually fuel is to be fabricated in Pakistan itself, and had Canada not, two years ago, stopped the shipment of equipment for fuel fabrication from Canada to Pakistan, the fuel for KANUPP would now be locally available. Considerable uranium bearing sandstones have been discovered in the northern areas of Pakistan in the Dera Ghazi Khan district and a survey of uranium bearing ores has been completed with the assistance of the International Atomic Energy Agency. Plans are in hand for their exploitation.

KANUPP feeds into the Karachi grid only 85 MW on average, and although its closure for long periods could be a source of irritation a 125 MW steam-powered generating unit is under installation, to be commissioned by July next. Another five gas-turbines each of 25 MW have also been urgently ordered for Karachi as a further reserve to be commissioned by July 1978.

The Pakistan government called the Canadian action arbitrary, adding that it violated three bilateral agreements on cooperation with Pakistan in peaceful uses of atomic energy. She found unreasonable the new conditions for which Canada was asking. One was that Canadian safeguards "will cover Pakistan's entire nuclear programme, not merely the nuclear facilities provided by Canada". Pakistan also found unacceptable the demand that even if "Canada should terminate all nuclear cooperation with Pakistan under the bilateral agreements, Pakistan shall nonetheless remain bound by its commitments under those agreements for the rest of the operating life of KANUPP". ☐

USA

Carter's energy problem

As America freezes energy matters grow more important. Colin Norman reports from Washington on the continuing search for an energy policy

It didn't take long for President Jimmy Carter to be brought face to face with the energy crisis. Within a week of taking office, he was forced to ask Congress for emergency powers to deal with a critical shortage of natural gas in parts of the eastern United States—a shortage brought about by an extraordinarily cold winter and several years' lack of a coherent energy policy. Though at this stage Carter can do little more than spread the shortage around, urge people to be less profligate in their use of energy and pray for warmer weather, the new Administration is promising to set things right in the long term with a comprehensive energy policy, a strong conservation plan and an overhaul of the federal energy bureaucracy.

Clearly, energy matters are going to keep the Administration busy, but at least it will have no shortage of outside advice. In that regard, a mammoth study now being put together by the National Academy of Sciences is likely to prove particularly influential. Due to be completed by June 30, the study is one of the most ambitious projects the Academy has yet undertaken. It involves some 250 people, sitting on a variety of panels and committees, who are now channelling their advice to a steering committee co-chaired by the ubiquitous Harvey Brooks, professor

Changing of the guard

- As the new Carter Administration settled into its first full week in office, selection of the second-level Presidential appointees began in earnest, but by the end of the week, none of the key science posts had been filled. The most important job, head of the Office of Science and Technology Policy (OSTP) and Science Adviser to the President, was still open following the departure of H. Guyford Stever on January 20. About the only sign of movement is that Carter has asked the Defense Secretary, Harold Brown, to draft a list of potential candidates for the OSTP post, a curious assignment since OSTP has little influence over defence matters, and most other Presidential appointments are being handled by a team in the White House.

As for the major science agencies, Donald Fredrickson, the popular director of the National Institutes of Health (NIH), had not heard by the end of last week whether he will be kept in his job. At a press conference, however, Joseph Califano, the new Secretary of Health Education and Welfare, promised to end the politicalisation of NIH, but he said he hadn't made up his mind about Fredrickson's future. Similarly, Robert Seamans Jr, has departed as head of the Energy Research and Development Administration, but no successor has been named. It is widely reported, however that Douglas Costle, a former staff member of the Congressional Budget Office has been chosen as head of the Environmental Protection Agency, to replace the already-departed Russell Train.

About the only major scientific appointment so far is that of Patsy Mink, a former Congresswoman from Hawaii, to be Assistant Secretary of State in charge of science and technology affairs. The post was once held by Dixy Lee Ray, the former head of the Atomic Energy Commission who is now Governor of Washington State, but she quit with a blast at Henry Kissinger because of his indifference toward her office. The selection of Mink, a lawyer, for a post which many people consider should be held by a scientist, or at least by somebody well versed in scientific matters, will not do much to enhance the status of the office.

- Meanwhile, at the other end of Pennsylvania Avenue, Congress is finally getting itself in gear for the new session. The plan to reorganise the committees of the Senate, which was drafted last year by a special select committee, has now been picked over and amended by the Senate Rules Committee and it is expected to be debated by the full Senate this week. Although several more changes are anticipated, the chief committee alignments for science and technology affairs are likely to remain virtually as proposed by the select committee.

The Joint Committee on Atomic Energy, which has already been cut up by the House, will be completely done away with, and all jurisdiction for energy policy and energy research and development will be placed in an expanded Interior committee, under the chairmanship of Senator Henry Jackson. Responsibility for biomedical research will be consolidated in a new Committee on Human Resources, based on the old Labor and Public Welfare Committee, which means that Senator Edward Kennedy's subcommittee will retain its jurisdiction. The rest of the responsibility for science and technology, including overall science policy affairs, will be placed in an expanded Commerce Committee. Senator Adlai Stevenson, who chaired the select committee, is expected to end up with chairmanship of the key Commerce subcommittee dealing with science and technology.

The only major change from the select committee's proposals is that jurisdiction over the National Science Foundation (NSF) will go to the Human Resources Committee. On the face of it, a decision to put NSF affairs into a committee which deals with health and welfare rather than into the Commerce Committee, which deals with science, may seem a bit illogical. The reason is that Kennedy, whose NSF subcommittee now handles NSF matters, is a member of the Human Resources Committee but not of the Commerce Committee, and he wants to hang on to his NSF jurisdiction. That's the way Congress works.

On the House side, the Science and Technology Committee, which has picked up responsibility for nuclear research and development from the Joint Committee on Atomic Energy, has been reorganised. Chairmanship of the subcommittee on Science, Research and Development, which deals with NSF, has been assigned to Representative Ray Thornton, a Democrat for Arkansas. Energy matters will be handled by two sub-committees, one of which will deal with fossil energy and nuclear fission and fusion, while the other will deal with solar and geothermal energy, energy conservation and basic energy research. Rep. Walter Flowers, a long-serving member from Alabama, claimed the chairmanship of the fossil and nuclear subcommittee, while Representative Mike McCormack, who was widely expected to get authority over nuclear matters, was left with the solar and geothermal subcommittee. Flowers' views on energy policy are little known, but his new post will give him plenty of visibility, a useful asset if, as expected, he decides to run for the Senate in 1978.

Colin Norman

of technology and public policy at Harvard, and Edward Ginzton, chairman of Varian Associates.

A tantalising preview of some of the conclusions likely to emerge from the study surfaced last week when the steering committee issued an interim report, essentially suggesting that energy use in the United States will grow at a slower pace than most other studies have anticipated. The committee suggests, moreover, that the country can adjust to a slower rate of energy growth without sacrificing economic or social goals.

If the Academy does indeed come up with such a conclusion, it would add considerable weight to the view put forward a couple of years ago by the Ford Foundation's Energy Policy Project, namely that energy growth can be gradually reduced over a decade or so to a very low rate, without either causing economic stagnation or radically altering people's lifestyles. The practical consequence of such a development—if true—would be that there may be much more flexibility in long term energy supplies than is generally realised; Mr Carter could, for example, carry out his campaign pledge to slow down the present breakneck pace of the breeder reactor programme, without running a large risk of causing massive economic disruptions toward the end of the century.

Further evidence of the growing acceptance of the view that energy growth is likely to slow markedly is also to be found in a meticulous study of the possible consequences of a nuclear moratorium, produced recently by the Institute for Energy Analysis (IEA), headed by Alvin Weinberg. The study predicts that total energy use in the year 2010 will lie between a low of about 118 quads and a high of about 159 quads, compared with 71 quads in 1975. It notes that "even our 'high' estimate . . . is much lower than most previously published estimates. If our estimates are valid, they could imply considerable rethinking of those elements of energy policy and energy R&D policy that are premised on higher overall projections."

Because of those lowered energy growth rates, the study estimates that the economic and environmental impacts of a nuclear moratorium (which would prohibit the construction of new reactors after 1980 but allow continued operation of reactors on line by 1985), though severe, would be smaller than is generally believed. It is assumed that most of the energy shortfall would be taken up by a switch to coal in the short term, with growing input from alternative sources such as solar energy, which would tend to increase the cost of energy by a total of between $31,400 and $42,000 million by the year 2010, the study estimates. That would amount to less than 1% of the gross national product each year. As for employment, the study predicts that a moratorium would result in the loss of about 50,000 jobs in the nuclear and related industries, but "the displacement caused by the moratorium would be temporary".

The environmental implications of a switch from nuclear to coal could be relatively severe. If the moratorium is coupled with limitations on oil imports, which would be likely, between 100 and 300 million more tons of coal would have to be mined each year in the period between 2000 and 2010 than would be the case without a moratorium. That would probably despoil larger areas of land for strip mines, increase atmospheric levels of carbon dioxide, and possibly increase pollution from sulphur dioxide, oxides of nitrogen, hydrocarbons, particulates and carbon monoxide. Increased use of pollution control technologies would, however, take care of some of the increases.

Though such impacts are relatively large, they certainly do not add up to the level of economic and social disruption predicted by the nuclear industry last year during the referenda in seven states on nuclear power.

The expectation that energy growth in the United States will slow down over the long term without precipitating economic stagnation, which drew sharp criticism when the Ford Foundation published its study, is therefore growing in acceptance. It means that President Carter may have more flexibility when he chooses his long range energy plans, but it won't help much over the next few years. Only some warmer weather would do that. ☐

AUSTRALIA

SCORE scores

Peter Pockley reports from Sydney on Australia's data collecting project for research expenditure

INACTIVITY on questions of priority and level of financial support for science in Australia has regularly been justified on grounds of a lack of reliable data on expenditure and manpower. In the early 1970s, the then Department of Education and Science started the first national data collecting operation and graced it with the acronym of Project SCORE (Survey and Comparison of Research Expenditures).

The project, however, was for some years plagued by problems of definition, management and staffing. But it did manage to grind out in 1973 a detailed set of figures for the financial year 1968–69 (the Australian financial year runs from July 1 to June 30, with the exception of the universities whose accounts run from January 1 to December 31—just one of the problems faced by the number crunchers). These figures came out just as inflation began to canter up towards the gallop of 1975 and 1976, and this, coupled with the long delay between year of study and year of publication, greatly diminished their value.

The Department of Science has now begun publishing the second set of Project SCORE data. These refer to the financial year 1973–74; being more recent, the project scores more useful points for the student and politician of science alike. However, the criteria used in collecting and processing the data are not entirely consistent with the 1968–69 figures because the project changed to following OECD prescriptions very closely. This makes international comparisons valid, but has negated any reliable and precise statements on national *trends* in expenditure and manpower which are so urgently needed for drumming complacency out of politicians. This will have to await the next publication planned for the financial year of 1975–76 (the Department now hopes to collect data at two-yearly intervals).

Project SCORE reveals that in 1973–74 the Gross Expenditure on Research and Development (GERD) amounted to 651 million Australian dollars (£1=$1.58). This represents 1.3% of the Gross Domestic Product (GDP) of $50,557 million for the year under study. The manpower involved in GERD was equivalent to 53,300 man-years, representing 0.9% of the Australian work-force of 5,867,700; 48% of the manpower effort was attributed to professional persons. There are, however, no figures for the supply and demand of professionals, nor are these available from other sources to confirm or deny the now popular belief in Australia of an oversupply of scientists and technologists at a time when overall unemployment is nudging 5% of the workforce.

Using OECD's definitions, 91% of GERD was spent in the natural sciences, and 9% in the social sciences and humanities, assuming all business enterprise expenditure to be in the former. Basic research took 28% ($184 million) of GERD, applied research

37% ($238 million) and experimental development 35% ($229 million).

Socio-economic objectives groups accounted for intramural expenditure as follows, assuming all expenditure in the business enterprise sector to be in the economic development group: economic development 59%, advancement of knowledge 26%, national security 9%, and community welfare 6%. These and other broad figures quoted are broken down into fine detail in Project SCORE's publications.

As a percentage of GDP, Australia's GERD (natural sciences only) was in the middle rank of OECD's 18 member countries for the calendar year 1973. Australia's figure, adjusted to that year, was 1.2%, compared with the USA 2.3%, UK 2.1%, Germany 2.0%, Netherlands 1.8%, Japan and France 1.7%, Sweden 1.6%, Belgium 1.3%, Canada and Norway 1.1%, Austria 1.0%, and New Zeland 0.9%.

Changes in Australian R&D expenditure in the five years from 1968–69 to 1973–74 are difficult to quantify because of the changed criteria. Project SCORE's published figures for 1968–69 put total GERD at $334 million, approximately half the 1973–74 figure of $651 million without taking inflation into account. If compared at constant prices, GERD has increased about 35%, but when measured as a percentage of GDP, the GERD figures are closely comparable.

The relative financial support of Australian R&D has remained fairly static over the five-year period between comprehensive data collections and analyses. The figures thus support the general contention from more superficial indicators that neither the Liberal nor Labor governments of recent times gave significantly more or less attention to science than to other areas of government finance. Using the superficial indicators for the period since 1973–74, it is at least certain that the relative standstill position has been maintained, and quite likely that there has been a significant slippage. For example, it is believed that the number of staff engaged on industrial R&D has dropped by as much as 40% over the two years from 1973–74. □

IN BRIEF

Gloomy energy report

In a sombre analysis released last week of projected global energy supply and demand to 1985, an OECD publication, *World Energy Outlook*, warns of a likely shortfall in oil supply that will mean either higher prices or shortages or both. The report, calling for a "transformation" and "revitalisation" of energy policies in industrial countries, urges them to increase indigenous energy production and improve conservation through a combination of financial measures, including investment incentives and use of the price mechanism, and regulations, embracing direct and indirect conservation measures and relaxed environmental requirements.

Brazilian deal developments

Reports in Brazilian papers suggest that Brazil is determined to resist pressure from the United States to change the terms of its controversial nuclear deal with West Germany. There are also signs that Argentina, which has its own nuclear ambitions, is proferring support for the stand. The reports follow others in the Brazilian press indicating a possibility that the US might consider supplying Brazil with nuclear fuel if it gave up that part of the German deal which includes both enrichment and reprocessing facilities.

The US Vice-President, Walter Mondale, apparently urged West Germany to restrain sales of nuclear technology when he visited Bonn last week as part of his diplomatic tour of key western capitals, and the possibility has emerged of including tighter curbs on trade in sensitive technology. Nothing specific was disclosed regarding the Brazilian deal, however.

Brazil has not signed the nuclear Non-Proliferation Treaty, and although the deal is permissible under the treaty, it is hardly in keeping with President Carter's intentions in the field of international trade in nuclear technology.

Safety bodies established

The UK Health and Safety Commission has announced the establishment of two more bodies which are to be part of the advisory committee structure that will help it discharge its obligations under the Health and Safety at Work Act. One is the Advisory Committee on Toxic Substances, and will be chaired by the Director of the Health and Safety Executive's Hazardous Substances Division; committee members include four with relevant expertise and the CBI and TUC have each nominated four. Attention will focus on new chemicals, but not nuclear materials, which a new committee to replace the Nuclear Safety Advisory Committee will examine.

The other committee is the Advisory Committee on Dangerous Substances, to be chaired by the Executive's Deputy Director. This will concern itself with flammable and explosive substances, and includes three expert members as well as three each from the CBI and TUC.

A third committee, the Medical Advisory Committee, is already functioning, and one of the approximately 18 industry-based bodies, for agriculture, is also in operation.

Finland nuclear report

Finland should put a sharp brake on its nuclear energy plans, says the report of an energy committee under the leadership of the Finnish Minister for Trade and Industry, Arne Berner. Finland's shaky economic situation demands that investments should be confined to projects which will increase employment and the development of exports, rather than to nuclear power: instead of the string of reactors which the government had thought of building around Finland's coast, the committee suggests that no new reactors should be built before 1985. If more energy is needed before then, it should come from non-nuclear sources and problems connected with nuclear energy should meanwhile be studied more closely.

Finland has four reactors under construction, at Lovisa and Olkiluoto, and the first is due to be loaded in a few months' time. By 1985 it is expected that they will be producing about 14% of the country's total energy consumption. Water power will supply about 23% (30% last year), with most energy continuing to come from oil, coal and peat.

There has been no general public debate about nuclear energy in Finland as there has been in Sweden, although groups living near the reactors have protested. The committee's report offers them no comfort. If Finland does build more nuclear power stations, it says, they will probably be sited near the existing ones.

Budgets announced

The 30-member Executive Board of the World Health Organisation last week recommended a budget for WHO activities in 1978 of $165 million. The World Health Assembly will consider the proposal at its 30th session in Geneva in May.

The Council of the European Space Agency, meeting in Paris, approved programme budgets for 1977 last week of 481.1 million units of account, some 3 million down on those proposed. The programmes include Aerosat, Ariane, Marots, Meteostat, OTS and Spacelab. The Ministerial Council holds its first meeting later this month and will discuss the level of resources for 1978–80.

news and views

Self-control by bacteriophage lambda

from a Correspondent

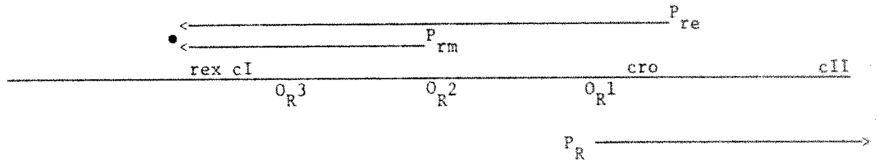
IT has been known for some time that the control of repressor synthesis in bacteriophage lambda is not a simple phenomenon. Such are the intricacies of the regulatory circuits involved in fact, that the subject was largely confined to European laboratories. Now, much of the lambda DNA sequence containing the *cis*-regulatory elements of repressor synthesis has been deciphered (Waltz *et al.*, *Nature* **262**, 665; 1976; Ptashne *et al.*, *Science* **194**, 156; 1976), and we can all take a crack at explaining the mechanism.

Shortly after infection of a sensitive *E. coli* by lambda, large quantities of repressor protein are synthesised. This repressor is translated from message initiating at a promoter, P_{re}, located several hundred nucleotide pairs to the right of the repressor structural gene, *cI*. Transcription from P_{re} depends on two phage products, *cII* and *cIII* which are not synthesised in an established lysogen. The maintenance level of repressor, about 200 repressor monomers per cell, is much lower than the infection level and depends on the activity of a different promoter, P_{rm}, located close to *cI*.

Transcription from P_{rm}, like transcription from P_{re}, is not constitutive. Repressor can be inactivated and its rate of reappearance measured over a long period. What is found is that, once a cell has lost repressor, it is very slow to resynthesise it. Since a prophage makes but two phage products, repressor and Rex, it would appear that one of these is responsible for maintaining the activity of P_{rm}. The experiments of Waltz *et al.* and Ptashne *et al.* demonstrate that repressor is its own inducer.

By the use of restriction endonuclease DNA fragments containing the *cI* control elements, the control of repressor expression can be shown *in vitro* at the level of mRNA synthesis. If the fragment is incubated with RNA polymerase, no transcription of *cI* is observed. Instead, all the transcripts initiate at the P_R promoter—the rightward promoter which is active in lytic phage development but repressed in a lysogen. Addition of a small amount of repressor to the incubation mixture turns off P_R transcription and activates *cI* transcription from P_{rm}. If large quantities of repressor are added, all transcription from the fragment is eliminated, including *cI*.

A simplified map of the control region is given below.



To the right of *cI* are three operators, or repressor binding sites, O_R1, O_R2 and O_R3. These sites are 17 base pairs long and are separated by spacers 3 to 7 base pairs long. They differ slightly in sequence and function from each other; O_R1 binds repressor more strongly than O_R2 or O_R3. Sequences recognised by RNA polymerase for rightward transcription, P_R, are located within O_R1 and O_R2. Repressor, by binding to these operators, prevents RNA polymerase from binding to P_R. O_R3 is not required to repress P_R; its role is to regulate the synthesis of repressor in lysogens. The control region displays a symmetry centred around the middle of O_R2, such that O_R3 contains a sequence similar to P_R, but with the opposite orientation. This is P_{rm}.

The sequence of the control regions from mutant phage have been obtained and support these assignments. Mutations in O_R1 or O_R2 destroy their capacity to bind repressor. A mutation located between O_R1 and O_R2 inactivates P_R, and a base change in the spacer between O_R3 and O_R2 destroys P_{rm}.

Speculating on the control of repressor synthesis on the basis of the DNA sequence we can see that the sequences of P_{rm} and P_R are not quite identical. This could explain why an additional factor, repressor for example, is required for P_{rm} activation. Moreover, the P_{rm}-promoted mRNA begins with AUG, and the codon corresponding to the amino terminus of repressor is found immediately adjacent to this triplet. Thus the P_{rm} message contains no leader sequence, hitherto thought to be essential for ribosome binding. This is not the case for the mRNA initiated at P_{re}, which carries the sequence GGTGAT. Since, under optimal conditions, P_{rm} and P_{re} are both very efficient promoters, the low level of repressor in a lysogen is due to inefficient translation of the P_{rm} mRNA, presumably because it is leaderless.

Unfortunately, it is still not obvious how repressor stimulates *cI* transcription. Perhaps P_{rm} competes with P_R for RNA polymerase, and the role of repressor is to block the binding of RNA polymerase at P_R. If this were the case, we would expect that mutations which reduce the efficiency of P_R as a promoter would relieve the repressor requirement of P_{rm}. Mutations isolated so far in P_R do not have this character. Alternatively, repressor bound at O_R1 may interact with RNA polymerase to stimulate directly its binding at P_{rm}. Ptashne notes that the distance between O_R1 and the initiation of *cI* transcription is the same as the distance between the start point of *lacZ* transcription and the site in the *lac* operon where the postive-control cyclic AMP binding protein interacts.

With either model, repressor synthesis in a lysogen will be negatively regulated at O_R3, where repressor protein competes with RNA polymerase for binding to DNA. The interplay of these positive and negative control circuits assures that the maintenance level of repressor will be confined to narrow limits.

The ability to isolate and sequence mutations in regulatory regions is certain to be tested again at other interesting lambda loci; notably at P_{re} and at the sites of action and recognition of the lambda control functions *N*, *Q*, *cII*, and *cIII*. □

Triassic extinction or Jurassic vacuum?

from Andrew Milner

In Charles Kingsley's *The Water Babies*, the author makes the observation that one cannot claim that water babies do not exist until one has seen them not existing. This little paradox is particularly pertinent to the fossils of terrestrial animals and what we deduce about them, particularly when they are absent from rocks of a given age.

If a taxon is absent from the fossil record for a given period of time, we logically assume that either it was genuinely absent or that it was present but that circumstances did not permit preservation. If the taxon or its descendants subsequently reappear as later fossils or living forms, we can be certain that the absence was due to non-preservation. If it never reappears the problem remains open and becomes: when did this taxon become extinct? Attributing a point in time to an extinction is only valid when the fossil record is comparably complete before and after the supposed event. Only if an animal is absent from an otherwise rich assemblage or if its ecological replacement is present, can one claim to have seen that animal "not existing".

The discovery of a temnospondyl amphibian in the Lower Jurassic of Australia announced by Warren in this week's issue of *Nature* (page 435) is a reminder of this. The Order Temnospondyli radiated during the Carboniferous to reach its greatest diversity in the Upper Carboniferous and Lower Permian. Many lineages became extinct during the Permian but others extended into and through the Triassic, and by the late Triassic several families remained, including the capitosaurs, brachyopids, metoposaurs and plagiosaurs. That the extinction of these families had taken place at the end of the Upper Triasic was virtually dogma. So much so that, as Warren notes, an earlier discovery of a fragment of Jurassic temnospondyl had been assumed to be a reworked bone from the Triassic simply because there was no precedent for it to have been any younger.

However, in order to have such fossils of Lower or Middle Jurassic amphibians or terrestrial vertebrates, one must first have vertebrate-bearing continental sediments of the same age and these are singularly scarce. The Upper Triassic of Europe, North America, Africa, India and China has produced an extensive range of terrestrial vertebrates including temnospondyl amphibians, procolophonids, chelonians, thecodonts, rhynchosaurs, dicynodonts, and early dinosaurs, crocodiles and mammals. By the Upper Jurassic, a very different assemblage of continental vertebrates is known from Europe, North America and East Africa. The chelonians, crocodiles, dinosaurs, pterosaurs and mammals predominate. The temnospondyls, procolophonids, rhynchosaurs, thecodonts and dicynodonts are all absent, presumed extinct, and in most cases their ecological replacements can be seen to be present. But when were they replaced? Did they become extinct synchronously on the Triassic–Jurassic boundary as a literal interpretation of the record would suggest? Or were they gradually replaced during the Lower and Middle Jurassic? Warren's amphibian suggests the latter possibility as do the tritylodonts which extend from Upper Triassic to Middle Jurassic.

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Several recent discoveries offer the hope that this problem will eventually be solved. A sauropod dinosaur has recently been described from the Lower Jurassic Kota formation of India (Jain *et al.*, *Proc. R. Soc.* **B188,** 211; **1975)** and new microvertebrate localities of Middle Jurassic age have been found on the Isle of Skye (Waldman and Savage, *J. geol. Soc.* **128,** 119; 1972) and in Oxfordshire (Freeman, *Science* **194,** 1053; 1976). We still do not have a rich assemblage of early Jurassic terrestrial vertebrates but further excavation of these localities and Warren's Queensland locality may provide us with enough material to characterise the Lower and Middle Jurassic faunas and hence determine the chronology of the turnover of terrestrial vertebrate taxa between the Upper Triassic and Upper Jurassic. ☐

Astrophysics in Boston

from James Pringle

> The Eighth Texas Symposium on Relativistic Astrophysics took place on December 13–17, 1976 in the Copley Plaza Hotel in Boston, Massachusetts. The proceedings of the Symposium will be published as a special issue of the Annals of the New York Academy of Sciences. Nearly a thousand astronomers and physicists were present for this biennial jamboree and there might have been many more but for the competing attraction of the mid-January American Astronomical Society which was taking place in Hawaii.

X-ray astronomy featured prominently in four of the ten sessions. In the session on X-ray astronomy itself R. Novick (Columbia University) reported on the first unambiguous detection of polarised radiation in X-ray astronomy. With the X-ray polarimeter on board OSO 8, he and his group have found that at X-ray energies the Crab Nebula is 22% polarised with a significance of some 15 standard deviations. This compares with 19% polarisation in the optical. The position angle of the polarisation is essentially the same in both energy bands. He presented marginal evidence for the polarisation of the X rays from the Crab pulsar itself, and indications that the swing in position angle of the X-ray polarisation direction through each pulse is comparable to that seen in the optical. In addition Cygnus X-1 may be polarised at the 3% level (a 2½ standard deviation result) and, at a higher level of confidence, Cygnus X-2 may be polarised at the 5% level. E. Boldt (Goddard Space Flight Center) reported on some further high time resolution observations of the black hole candidate X-ray source Cygnus X-1, which continues to display X-ray activity on timescales as short as a few milliseconds. He gave tentative evidence that there may be a repetitive occurrence in these millisecond bursts with a timescale of about 10 ms and speculated that this might be identified with material falling within the last stable circular orbit for a black hole of some 17 solar masses.

Ken Pounds (University of Leicester) gave a preliminary report on the catalogue of X-ray sources being prepared from data from the Ariel V satellite and on a programme of optical identification of previously unidentified high galactic latitude sources (UHGLs for short). He argued that most of the 50 or so UHGLs in the UHURU catalogue of X-ray sources are now accounted for and so the UHGL mystery is solved, although the attentive in the audience noted that he had at the onset reported some 45 new UHGLs found by Ariel V.

The discovery of bursting behaviour

at X-ray energies is becoming increasingly common, although the implied link in the title of the session "X-ray bursters and Globular Clusters" was rather controversial. Walter Lewin (Massachusetts Institute of Technology) argued strongly that, although two of the bursting sources were almost certainly within globular clusters, the connection between the others and globular clusters was by no means proven and that the distribution of bursters within the galaxy is unlike that of the globular clusters. Jerry Ostriker (Princeton University) disagreed and argued, perhaps less convincingly but no less strongly, that the two distributions are remarkably similar. Lewin went on to discuss individual bursting sources of extraordinary complexity from MXB 1743-29 which bursts only once a day or so to MXB 1728-34 which is known as the 'rapid burster' producing bursts of X rays some 5,000 times a day. As more and more details of the bursting behaviour accumulate, it becomes more difficult for theorists to find a convincing explanation of the phenomena. Don Lamb (University of Illinois) was given the problem of finding something intelligent to say on the theoretical aspects of the bewildering observational results. He discussed a variety of possibilities including bursts of nuclear burning on the surfaces of neutron stars, and accretion events involving material held up by the magnetosphere of a neutron star sporadically going unstable and crashing to the surface.

Part of the limelight at the session on Neutron Stars was stolen by J. Trümper (Max-Planck-Institut, Munich) who had flown in especially to announce data obtained in a balloon flight looking at Hercules X-1 in the energy range 20–120 keV. It had been thought previously that the X-ray spectrum of Hercules X-1 did not extend significantly above about 30 keV. Trümper reported that the source had been seen well above 30 keV and the X-ray pulses (with a period of 1.24 s) were seen up to energies as high as 70 keV. Moreover, he presented evidence for an emission feature in the spectrum above 40 keV which he interpreted as an emission line due to cyclotron radiation at an energy of 53 keV—there is no other likely reason for the production of an emission line at this energy. If this is true, it implies that Hercules X-1 is indeed a magnetised neutron star and that the magnetic field is in the region of 2–5×10^{12} gauss (depending primarily on which harmonic of the gyrofrequency the emission is supposedly produced). Following this talk, Leventhal (Bell Laboratories) reported on γ-ray balloon observations of the Crab Nebula made to detect γ-ray lines. He gave evidence for a strong γ-ray line at an energy of 400 keV and speculated that this might be the 510 keV electron–positron annihilation line redshifted from the surface of the Crab pulsar: the redshift would imply that the pulsar is a neutron star of mass 1.4 solar masses, a value highly favoured on stellar evolutionary grounds.

Low mass X-ray binaries were discussed by Anne Cowley (University of Michigan) in the session on Close Binary Stars. The most intriguing source recently discovered to emit X rays is the star AM Herculis. This was already known to be a "nova-like variable", implying that a large amount of flickering is present in the optical light. It has since been found to display a 3-h period, probably due to orbital motion, in optical light and in radial velocity and to emit soft X rays which vary with the same period. Most remarkably, the optical light is highly circularly polarised (5–10%) with the degree of polarisation changing on the same 3-h period. It seems that this polarised light can only be due to cyclotron emission, implying the presence in the system of a magnetic field with a strength of over 10^8 gauss.

The second morning was devoted to quantum theory in strong gravitational fields. Stephen Hawking (Cambridge University) spoke on black holes and unpredictability. Inside a black hole, there is always a singularity which is a place where space and time come to an end and all the laws of physics break down. Provided the black hole can prevent any information about the singularity from leaking out, however, any observer not in a black hole would not be affected by the breakdown of predictability which occurs at the singularity. This "cosmic censorship" is endangered by Hawking's discovery that quantum effects allow particles and radiation to tunnel out of black holes. This radiation has an added degree of randomness over and above that associated with quantum mechanics. Equivalently, he concluded, one could say that "new random information is entering our universe from regions (either singularities or 'worm holes' leading to other universes) about which we have no knowledge".

In the session on Gravitational Theories Larry Smarr (Harvard University) gave a talk with the ominous sounding title "Computer Generated Space-Times". He reported on a continuing effort to solve Einstein's field equations in their full time-dependent form by means of numerical methods. One of the aims of this research would be, for example, to study the collision of two black holes with a view to discovering how much energy is radiated away in the form of gravitational radiation.

Irwin Shapiro (Massachusetts Institute of Technology) opened the session on quasars with a talk entitled

Membrane-bound proteins

from I. B. Holland and R. H. Pritchard

MEMBRANE-associated proteins are hard to study. They will probably be insoluble in aqueous solvents, there is often no simple assay for them and consequently they will probably defy purification. Studying their function may not be a simple matter either. The way a protein which facilitates transport of a small molecule across a membrane works will need to be investigated with the protein *in situ* or attached to an artificial membrane. Silhavy *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **73,** 3423; 1976) have found a new way of studying these proteins using the sophisticated box of tricks available to the *Escherichia coli* geneticist. They selected rare variants of this bacterium in which a gene coding for a protein involved in transport of maltose, and thought to be membrane bound, was fused to a gene coding for an easily assayable enzyme (β-galactosidase). The fused genes made a fusion protein which had the NH_2-terminal end of the transport protein and the COOH-terminal end of β-galactosidase. This hybrid protein had β-galactosidase activity which could be used to show that it was located in the cytoplasmic membrane. Thus an enzyme normally found in the cytoplasm had been carried to the membrane suggesting that the maltose transport protein is indeed membrane bound and that its COOH-terminus is not needed for this binding to occur.

Silhavy *et al.* hope to use their technique to investigate the structural features of proteins which lead them to associate with membranes. Their experimental strategy may be equally useful in identifying otherwise non-assayable proteins and for histochemical studies.

Unfortunately, in organisms with underdeveloped or emerging genetic technologies it will not be possible to engineer the variants required to make fusion proteins. This may only be a temporary difficulty in the exploitation of a promising idea.

"Apparent Superrelativistic Expansion of Quasars: Evolution or Revolution?". He reported on further radio VLBI (very long baseline interferometry—with baselines extending across the Atlantic) observations of quasars which show source structure down to an angular site of 0.001 arc s or less. Almost all the sources observed show time variations and some 10–15% of them show components which are separating from each other with apparent velocities greater than that of light. In the quasar 3C279 two compact radio sources are seen about 8 parsecs apart which appear to be flying apart at a speed some 25 times that of the velocity of light. There is as yet no convincing explanation of this phenomenon.

Dealing with theories of quasars, Martin Rees (Cambridge University) argued strongly that the observational evidence, including the VLBI observations presented by Shapiro, all tended to indicate that quasars are powered by a single massive object in the nucleus of a young and distant galaxy, and further that the most likely candidate for such an object would be a massive black hole. He described how such a black hole could provide the huge luminosity required (about 10^{46} erg s^{-1}) by slowly devouring the dense cluster of stars that is likely to be around it.

The final afternoon session was devoted to a discussion of galaxies. Harry van der Laan (Leiden Observatory) presented the latest observations of double radio galaxies by the Westerbork telescope. Polarisation observations provide a means of delineating the global structure of the magnetic field in the giant radio lobes: in general the field lies along any elongated features extending over very large distances and lies perpendicular to the intense radio hot spots at the outer edges of the radio lobes, as if the spots act like snow ploughs gathering field as they expand from the centre. He also discussed statistical evidence on the way the power houses which produce the double radio sources are distributed among elliptical galaxies. Somewhat surprisingly the evidence suggests that although the more massive (and brighter) elliptical galaxies are more likely to be endowed with an active power house, the size of the power house provided is independent of the size of the galaxy in which it is placed.

E. Salpeter (Cornell University) presented some 21-cm observations of galactic rotation curves using the newly re-surfaced Arecibo disk to extend the measurements out to radii of some 100 kpc from the centre. The graph of rotation velocity against radius should remain roughly constant if there is a massive unseen halo of faint stars (or even Jupiters) surrounding the optical galaxy, but should otherwise decrease with increasing radius. He found that the only galaxies observed which did not show evidence for a massive halo were those which had a close enough binary companion which would by now have stripped away the outer parts of any such halo, had it ever existed.

Alar Toomre (Massachusetts Institute of Technology) ended the conference with a light-hearted and amusing talk showing how tidal interactions between galaxies can produce spiral structure tantalisingly similar to that observed. He finished by showing how if a perturbing galaxy scores a "bulls-eye" by passing through another galaxy sufficiently close to the centre, the perturbed galaxy shows a bright, expanding ring structure. □

Animal behaviour and earthquake prediction

from John M. Logan

A conference on Animal Behaviour and Earthquake Prediction, sponsored by the United States Geological Survey, was held in Menlo Park, California on October 23–24, 1976.

CAN variations in animal behaviour help to predict earthquakes? As early as 100 AD Pliny advocated its use, but until recently the topic has received little serious scientific attention. Efforts now being made by the Chinese have, however, stimulated renewed interest in the topic. It has become especially relevant as other postulated precursors such as v_p/v_s ratios, electrical resistivity, radon content, electromagnetic variations and changes in the electrostatic field are either being questioned or are in the developmental stages.

The first question to be asked is whether there is any scientific basis for the popular reports of abnormal behaviour of animals, birds, fish and reptiles before earthquakes? If so, can animal behaviour then be considered as a reliable predictive indicator; what are the physical or chemical factors causing the anomalous behaviour; what appropriate research programme can be suggested to investigate behaviour patterns and their associated stimuli; and finally, can the biological indicators eventually be replaced by physical receivers? It became apparent at the outset that the various skills of geologists, geophysicists, and biologists would be needed. Thus, possibly the most diverse group of scientists ever to discuss the problem of earthquake prediction was brought together at the conference.

Anecdotal reports by non-scientists from many parts of the world contain many similar observations of animal behaviour before earthquakes. These observations come from people who could not possibly have had contact with each other or have read reports from other areas. This lends credibility to the observations, and provides a large body of evidence supporting the contention that animals do sense something before earthquakes. Apparently the response of animals is not restricted to a single species or genus, but spans a very wide spectrum of biological forms which suggests that there is either more than one stimulus or a universal one that can be detected by a wide variety of organisms. Furthermore, with the possible exception of reports of snakes coming out of the ground in mid-winter, few of the responses reported are truly 'abnormal' or extreme. Most involve normal forms of activity which take place either at elevated levels or at unique times. Thus, the term "abnormal behaviour" is somewhat misleading.

D. Mead (San Jose Mercury) witnessed the unusual behaviour of a cow immediately preceding one of the aftershocks at Oroville, California, and was told that similar actions had been observed before previous earthquakes. Soon he felt the aftershock. Most convincing of all was the report by H. Kraemer, B. Smith, and S. Levine (Stanford University) on the behaviour of chimpanzees preceding a magnitude 3.7 event. This is one of the few scientifically based studies available, and the correlation between the chimpanzees' behaviour patterns and the earthquakes is very convincing.

Precursive physical phenomena associated with earthquakes that might serve as stimuli for recognisable variations in animal behaviour were discussed at some length. The important point was made that earthquakes have a number of causal mechanisms rather than any single one. Fracture of rock along a new fault, breaking of asperities along existing fault zones, fracture of gouge zones, material and other instabilities along fault surfaces all may release seismic energy. It is possible that not all earthquakes are preceded by the same precursive events, and it follows that potential stimuli for perturbations in animal behaviour may not be present in all earthquakes.

It was pointed out that observed changes in air pressure, gravity, elevation and tilt all fall within the normal range experienced by animals throughout the day and are doubtful stimuli of behavioural variation. D. Hill (US Geological Survey) demonstrated that acoustic emissions could only be considered a potential stimulus if they were centred within a few hundred feet of the surface and almost beneath the animal. Observed variations of a few gammas in electromagnetic signals associated with earthquakes also seem unlikely to produce significant variations in biological responses, as these are an order of magnitude less than the normal diurnal variations experienced by the animal if it stands still; if it moves about, it will experience changes of hundreds of thousands of gammas in a single day. Changes in near surface water level may provide a possible explanation for some behaviour such as snakes leaving their holes in midwinter, but such changes certainly cannot be cited as a stimulus for the majority of the reported behavioural perturbations. The two remaining areas —changes in the electrostatic field and gas emissions before earthquakes — have not been investigated in great detail and both may be potential stimuli. The conclusion reached was that either electrostatic (or other electrical) effects are the primary causes of unusual animal behaviour, or some other effect not yet investigated (smell?) is operating.

A number of examples of the extreme sensitivity of biological systems to small changes in their environment was reported. Most of the anecdotal reports of animal behaviour can probably be classified as startle responses and are probably not learned. Stimuli producing the startle response may simply exceed some noise level or may be due to variations of the pattern of stimuli within the normal range experienced by the animal. If the latter is true, then detecting variations in patterns of stimuli will prove much more difficult than detecting a critical level of the signal.

In conclusion, it was pointed out that an earthquake prediction programme making use of animal behaviour may take two forms. The first is the recognition of variations in animal behaviour preceding earthquakes and its use as a predictive tool without understanding the mechanics of the process. This empirical approach, if successfully developed would satisfy the immediate need of earthquake prediction. Research could, and probably should, be oriented to this end. The second approach involves an investigation of the stimuli involved, which could lead to direct monitoring of the physical signal without the intermediate step of observing animal behaviour.

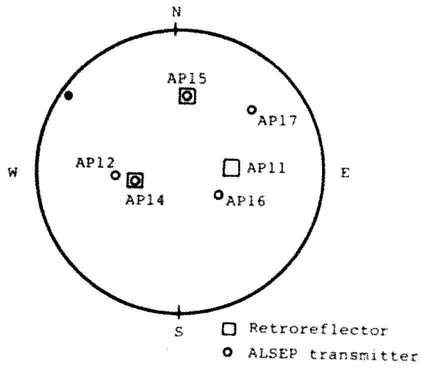
The interdisciplinary nature of the problem is extremely stimulating. Future research will clearly involve geologists, geophysicists, and biologists. I feel that the conference has successfully identified the problem, asked the pertinent questions and posed a number of hypotheses. Future work, it is hoped, will bring this to a level of scientific benefit to mankind. □

Moon measurements

from A. H. Cook

Placing optical retro-reflectors and radio transmitters on the surface of the Moon has proved to be one of the most significant and lasting achievements of the Apollo programme. The retroreflectors of which three were placed in the US programme and one (of French construction) in the USSR programme, have been used to reflect pulses of light emitted by laser transmitters on the Earth so that the round trip travel time of the pulse and hence the distance between a point on the Earth and one on the Moon can be measured very accurately, to within about 30 cm at present. So far almost all the measurements have been made from the Macdonald Observatory in Texas (Bender *et al., Science* **182,** 229; 1973). The radio transmitters have been observed by interferometers with very long baselines (VLBI) which are sensitive to the rotation of the group of transmitters about the line joining the centres of the Earth and the Moon (King *et al., J. geophys. Res.* **81,** 6251; 1976). Accuracies corresponding to between 1 and 10 m on the surface of the Moon are achieved.

The distance between a point on the Earth and one on the Moon depends on three factors: the motion of the Moon in her orbit about the Earth, the rotation of the Earth upon its axis and the oscillations of the Moon about her centre of mass. The three factors can be separated (the separation would be sharper if more laser observatories were in operation on the Earth and especially one in the southern hemisphere) and in consequence a great deal of physics can be studied. Thus the orbit of the Moon contracts because of the friction which damps the tides upon the rotating Earth; it may also be possible to check certain rather general relativistic theories. It is hoped that precise measurements of the relative positions of terrestrial observatories will lead to direct observation of movements of tectonic plates and to improved understanding of the very imperfectly known variations in the vector of the Earth's spin. In astronomy improved measurements of the Moon may lead to a better fundamental catalogue of star positions.



Retro-reflectors and radio transmitters used for analyses of lunar librations (King *et al., op. cit.*). AP denotes the Apollo mission which carried the equipment.

One of the most exciting consequences of the observations is the improved knowledge of the lunar librations. The Moon moves about the Earth in such a way that, seen from the Moon, the Earth remains almost but not quite in a fixed position relative to the axes of inertia of the Moon. Now the Moon has three unequal moments of inertia and also there are important terms of high harmonic order in the gravity field, and in consequence when the Earth moves with respect to the Moon it exerts torques which cause the Moon to rock about the lunar centre of mass. That rocking motion is the physical libration of the Moon. The amplitudes are small and difficult to observe visually from the Earth but by using laser ranging to the Moon the motion has already been observed in detail with high accuracy. The interest of observations of the libration lies in the fact that the libration depends on the moments of inertia of the Moon and when the observations of libration are combined with those of the second harmonic coefficients of gravity as obtained from artificial satellites about the Moon (Gapcynski *et al., Geophys. Res. Lett.* **2,** 353; 1975), then the three moments of inertia may all be obtained separately (Williams *et al., The Moon* **8,** 469; 1973). The moment of inertia of a celestial body is a key property in the study of its constitution because it depends on the way the density varies from the centre outwards. If the density were constant the moment of inertia in non-dimensional form would be 0.4 exactly, and when the density decreases towards the surface, the non-dimensional moment of inertia is less. The value for the Moon now seems to be close to 0.392, less, but not much less than 0.4. This is a stringent constraint on models of the lunar interior and because the value is so close to 0.4, it is most desirable to have it measured with as great an accuracy as possible.

It is here that the radio transmitters come in. Because of the fact that the technique of VLBI measures the rotation of the group of transmitters about the line of centres of the Earth and the Moon, it is insensitive to the Earth's rotation and to the Moon's orbital motion, and so leads to a very clear separation of the librations from other motions. The publication (King *et al.*, *op. cit.*) of the results obtained by combining lunar laser ranging with VLBI observations of the lunar radio transmitters is thus a landmark in lunar studies. The remarkably low uncertainty of 0.003 is attributed to the estimate of 0.392 for the value of the non-dimensional polar moment of inertia. It is also important that certain of the higher gravitational harmonics can be derived from the librations because there are considerable uncertainties in their derivation from the orbits of artificial satellites alone.

Clearly a great advance has been made in our knowledge of lunar dynamics in the past few years, and that in itself emphasises how much there is still to learn about the dynamics of the Earth. As already mentioned, one of the most important steps forward would be to obtain data from a lunar laser ranging observatory in the Southern Hemisphere, and there are indeed now good prospects of obtaining such data as a result of cooperation between US and Australian groups, in which British workers are also participating. As ever when new possibilities of precise measurements are realised, a new world of knowledge seems to be opening. □

Cheap uranium from bacteria?

from a Correspondent

An International Atomic Energy Agency research coordination meeting on the bacterial leaching of uranium ores was held at the University of Warwick on December 13–15, 1976 under the chairmanship of Professor Donovan Kelly, of the Department of Environmental Sciences, University of Warwick. The meeting was formally hosted by the UK Department of Energy.

During the 1960s it was found that uranium could be recovered from the acidic waters running through dumps of uranium mining wastes or from water hosed on to the walls of worked-out uranium mines. This solubilisation of uranium was subsequently found to be due to the activity of a specialised group of unusual bacteria called *Thiobacillus ferrooxidans*, which obtain energy for their growth from oxidising iron from the ferrous to ferric form in very acidic environments. These bacteria are always found in acid mine waters when pyrite (iron sulphide) is present in the ore being mined. They attack the pyrite and produce sulphuric acid and ferric iron, which in turn chemically converts the insoluble uranium ore into soluble uranyl sulphate. The possible economic importance of this process as a potentially cheap means of uranium recovery was early recognised by the IAEA, whose headquarters are in Vienna, and which has supported research and development into the bacterial leaching of uranium for a number of years.

The meeting at Warwick University was the third coordination meeting (earlier ones were in Ankara in 1972 and Vienna in 1974), held to review recent developments and to review the present status of the whole subject after some ten years of active work in many countries. Scientists from Austria, Canada, Finland, Hungary, India, Romania, South Korea, Yugoslavia and the UK and USA took part. Much of the work discussed had been financed by the IAEA and ranged from the basic microbiology of the process over economic aspects to the safety and control of uranium recovery.

With the likely demand for uranium for nuclear power generation in the Western World alone between 1976–2000 standing at 4 million tonnes of U_3O_8, mining companies have increasingly been looking at ways of recovering uranium from ores containing progressively less uranium, and to ways of recovering the small amounts of uranium left in the wastes from the processing of richer ores. Bacterial leaching provides just such a process, since the bacterial activity is as effective in removing essentially all the uranium from a 0.03% U_3O_8 ore as from a 0.3% ore, without necessarily being more costly, whereas the difference between these ores for conventional extraction processes is sufficient for the lower grade one to be uneconomic to work at acceptable prices. Bacterial leaching thus enables countries with only low-grade 'uneconomic' ores to exploit their natural resources cheaply and of course can also be used for low cost recovery of uranium from high-grade ores.

Since the IAEA programme began, the state of the art of bacterial leaching has progressed from minimal understanding and exploitation to the current state of having a rather full understanding of the fundamental microbiology and biochemistry of the process, to have proved large scale pilot plants and built at least one commercial scale plant. The latter, reported at the meeting, will become operational in Canada in 1977 and will produce a million pounds of U_3O_8 a year, using 3,000 tonnes of crushed ore per day.

The meeting discussed the basic studies of growth and physiology of the iron bacteria, largely carried out by Professor Kelly's group, the immunological characterisation studies performed in Korea, and work on the control of leaching activity and pilot plant operation in Romania, Yugoslavia and India. Much time was devoted to the evaluation of large scale plants, their engineering design, efficiency, responses to environmental factors such as seasonal temperature variation, supply of oxygen and of other nutrients, and possible hazards in the operation of the extraction systems. Britain has been active in the design and evaluation of practical leaching systems and the use of the bacteria to generate ferric iron from various sources to be used in leaching. A report on the work of the Warren Spring Laboratory in this field was received with considerable interest. The very large scale alkaline leaching of uranium in Hungary was reported in full and provided further information of basic use in plant design.

The conclusions of the meeting, reported to the IAEA, included the recommendation that bacterial leaching of uranium should be developed and encouraged as a cost-competitive method of recovering uranium, involving smaller capital and running costs than conventional methods, with the incalculably valuable bonus of enabling treatment of ores otherwise prohibitively costly to treat. It was foreseen that work in this field would proceed to progressively more controlled processes and that the areas requiring further work could be detected by constructing the realistic process flow sheets and mathematical models that work to date had made feasible.

A long-standing problem in many mining operations is the release of polluting acids and metals into the environment. One conclusion of this meeting was that controlled exploitation of leaching of waste rock from conventional mining operations was to be encouraged and exploited not only to recover uranium and other metals, but to minimise the likelihood of environmental pollution by uncontrolled natural leaching. It is clear that the economic, biological, sociological and engineering aspects of the bacterial recovery of uranium will occupy many workers for years to come. □

review article

Targeting of drugs

Gregory Gregoriadis*

Recent work suggests that specific association of drugs with their targets could be achieved by means of a versatile carrier capable of transporting drug molecules from the site of application directly to the site of action.

An important prerequisite for success in the application of pharmacologically active agents is specificity. Unfortunately, most agents interact with non-target sites as well. This is well illustrated in cancer chemotherapy of which the slow progress can be attributed to the failure of cytotoxic molecules to act on malignant cells only. Poor selectivity can often be worsened by the failure of agents to enter the target area. Thus, in certain cases of drug resistance development, cells (for example malignant cells) become impermeable to drugs, and in antimicrobial therapy antibiotics have little or no access to intracellular sites harbouring microorganisms. Access of drugs to the target can also be prevented because of gross anatomical barriers. Penetration of the blood–brain barrier by enzymes for instance would be desirable in the treatment of some lysosomal storage diseases[1].

There are at least two options open to us in pursuing optimal drug action. (By drugs I mean not only conventional drugs, enzymes and proteins used in medicine, but also substances used in biological research.) In the conventional one, efforts are directed towards the creation of specialised molecules. Usually a therapeutically profitable target–agent relationship is far from ideal, and undesirable side effects are almost always present. The alternative could prove simpler: functional molecules, not necessarily target-specific, could be transported by a carrier to the site of action and released to perform their task. Transport should be effected in isolation from the biological space between the site of application and the site of action because this might be important in where agents are either prone to inactivation or detrimental to the non-target space in the host. The carrier itself should be non-toxic, biodegradable and of the appropriate shape and size to accommodate a wide variety of substances. It should preferably ignore, or be ignored by, irrelevant areas and have a pronounced affinity for, and easy access to, the target site within which there should be a mechanism for the release of agents from the carrier. The latter, having accomplished its function, would then be disposed of.

In searching for the ideal carrier, one is tempted to look for systems which can intrinsically 'recognise' the target (Table 1). Thus, antitumour drugs complexed to antibodies have been shown to improve cytotoxicity to malignant cells possessing antigens corresponding to the immunoglobulin carrier[2]. As binding to the immunological determinants of the membrane is believed[80,81] to initiate endocytosis, the action of the antibody-bound drug is likely to be lysosomotropic (Fig. 1*b*). In spite of its appeal the antibody carrier approach is associated with a number of difficulties among which the isolation of target specific antigens for the producton of antibodies and the purification from these of immunoglobulin molecules specific for the antigen, are prominent. In addition, drugs attached to immunoglobulins may mask[83] the antigen recognition site of the protein or alter its tertiary structure and render it prone to rapid removal by the reticuloendothelial system. Assuming that target–carrier contact can be made one may ask whether the immunoglobulin–drug complex will allow the active moiety (drug) to perform its task. Severance of the bond (usually an ester) between carrier and agent may be necessary and as there could be a variety of linkages to suit a variety of agents, there should be a corresponding number of esterases. Other difficulties include inhibition of the immunoglobulin–antigen (target) interaction by circulating antibodies raised by the host against the antigen and also the possibility of allergic reactions to the carrier itself.

An interesting group of macromolecular carriers, only modestly pursued, is asialoglycoproteins (Table 1). These exhibit *in vivo* a highly specific affinity for the hepatic parenchymal cells[84] by which they are taken up into the lysosomal apparatus[85]. Using asialofetuin it was possible[10] to direct to the liver lysozyme and albumin cross-linked to the protein, and a similar use could perhaps be made of agalactoglycoproteins and ahexosamine glycoproteins which possess specificity for the liver and kidney respectively[86]. Other potential carrier molecules are trophic hormones, lectins[82] and some lysosomal enzymes[87]. In contrast to antibodies, many of these substances are readily available and complications in target recognition are less likely to occur *in vivo*. However, they lack the breadth of target choice of immunoglobulins as a group.

In a different approach for target selectivity advantage is taken of the ability of a number of cell types (for example phagocytes and certain malignant cells) actively to endocytose macromolecules. Among the latter (Table 1) a notable example[15] is DNA, to which cytotoxic drugs such as daunomycin and adriamycin bind tightly upon contact. Experiments with DNA-bound daunomycin and adriamycin have shown that reduced toxicity[88] of the bound drug and, presumably, increased uptake of the DNA–drug complex by tumour cells contribute to cures of tumour-bearing animals[15,17] and to remissions of certain malignancies in man[16]. In another example[11], β-amanitin coupled to albumin was found to induce *in vivo* selective damage of the sinusoidal liver cells and kidney proximal tubule cells, both very actve in the endocytic uptake of proteins. As with most macromolecules discussed, these lysosomotropic (Fig. 1) carriers can be vulnerable to the biological milieu (for example

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Table 1 Drug carriers in biology and medicine

Carrier	Associated drugs
Macromolecules	
Immunoglobulins[2]	Methotrexate[3], daunomycin[4], adriamycin[4], chlorambucil[5,6], radioactive iodine[7], diphtheria toxin[8], glucose oxidase[9]
Desialylated glycoproteins	Lysozyme[10], albumin[10]
Albumin	Amanitin[11], phalloidin[12], deoxycholic acid[13]
Fibrinogen	Dichloroethylphosphoramide[14]
Deoxyribonucleic acid	Daunomycin[15,16], adriamycin[17]
Dextran	Insulin[18], noradrenaline[18], amphetamine[18], novocaine[18], coenzyme B_{12}[18]
Cells	
Erythrocytes	β-glucosidase[19], β-galactosidase[19], glucuronidase[20], asparaginase[21], glucose oxidase[22], methotrexate[23], adriamycin[23]
Leucocytes	'Corrective' enzyme[24]
Hepatocytes	Uridine diphosphate glucuronyltransferase[25]
Synthetic systems—non-biodegradable[26]	
Nylon semi-permeable microcapsules	Carbonic anhydrase[26], uricase[26], asparaginase[26], urease[26], catalase[26]
Polyacrylamide gel	Asparaginase[27]
Liquid surfactant membranes	Urease[28]
Glass beads	Uricase[29]
Synthetic systems—biodegradable	
Albumin microspheres	6-mercaptopurine[30], actinomycin D[31]
Multiple oil emulsions	Naltrexone[32]
Lactic acid polymers[33]	Cyclazocine[33]
Ufasomes (unsaturated fatty acid spheres)[34]	
Liposomes[35]	Lysozyme[36], amyloglucosidase[37,38], neuraminidase[39,40], α-mannosidase[41], glucocerebroside:β-glucosidase[42], hexosaminidase A[43], asparaginase[44], peroxidase[45-47], glucose oxidase[48], lectins[49], immunoglobulins[46,50-53], insulin[54,55], diphtheria toxoid[56], Poly(I):Poly(C)[57], actinomycin D[58-61], 5-fluorouracil[62], methotrexate[63,64], bleomycin[48,50,65], cytosine arabinoside[66], 8-azaguanine[67], mechlorethamine[68], bichloroethyl nitrosourea[68], colchicine[69], penicillin G[70], steroids[71,72], cyclic AMP[73], pepstatin[74], glutamate[75], nitroblue tetrazolium[76], EDTA[77], DTPA[77], TcO_4[78,79]

Association of drugs with carriers can be effected through covalent, hydrophobic, hydrogen or other types of bonding. In synthetic systems passive entrapment of drugs is also possible. Only representative bibliography is shown for antibody[2], non-biodegradable[26] and liposomal[35] carriers. For more information see respective references.

blood nucleases in the case of DNA) and are limited in both range of agent accommodation and target choice.

A number of three-dimensional systems (Table 1) have been proposed as alternatives to the 'linear' macromolecular carriers. Depending on their composition, such systems can be biodegradable or non-degradable. Of the latter, a well-known example is the nylon semipermeable microspherule[26] within which various enzymes have been entrapped and found capable of acting on inwards diffusing substrates. Like most non-biodegradable carriers, however, these are unsuitable for parenteral (chronic) use. Of the biodegradable systems on the other hand, whole cells could prove of considerable value. Erythrocytes (Table 1) for instance could be useful in delivering agents to the reticuloendothelial system[19] into which these cells eventually find their way. Furthermore, and assuming that their life span *in vivo* is largely unchanged by the procedure of agent entrapment, erythrocytes could serve as devices for the slow release of drugs into the circulation. Alternatively, undesirable substrates could enter erythrocytes to be degraded by entrapped enzymes[21]. While erythrocytes are limited in both range of agents which they can carry and target accessibility, this is even more so for other cells (for example leukocytes, hepatocytes) which function as vehicles of agents they contain naturally (Table 1). Nonetheless, Brady's suggestion[89] of transporting enzymes past the blood-brain barrier in leukocytes (known to percolate through the cerebral tissue after emigrating from post-capillary venules) may render such cells valuable therapeutic tools.

How realistic then is the concept of an effective multipurpose carrier? For reasons outlined earlier, it is apparent that there is indeed a requirement for a three-dimensional system capable of containing agents within a well-protected space and amenable to modifications, both structural and functional. In retrospect, it seems unlikely that any other natural materials would have been as appropriate building blocks for the carrier as insoluble polar phospholipids. These, when confronted with water, form highly ordered assemblages of one or several concentric lipid bilayers known as liposomes[90,91] and which by the use of simple techniques[92] can accommodate a wide variety of water soluble substances (Table 1). Alternatively, lipid soluble materials including the hydrophobic regions of macromolecules such as proteins, can be inserted into the lipid space in the bilayers[93]. Several of the physical properties of liposomes can be varied at will: size (radius) can be adjusted from about 12 nm for monolamellar liposomes to up to several microns for the multilamellar version, and a negative or positive surface charge can be imposed by the incorporation of charged amphiphiles[92]. Control of permeability to entrapped substances, and of stability is also feasible by the addition of a sterol or other lipids into the liposomal structure[92]. Finally, manipulation[35] of the surface characteristics of liposomes (see below) will enable them to recognise specific cells.

Following the demonstration[94,37] that injected liposomes control both the rate of elimination from the circulation and tissue distribution of entrapped agents, a considerable amount of work has been carried out in regard to the possible role of liposomes as carriers in biology and medicine[35]. For example, intravenously given liposomes by and large remain intact in the blood and entrapped non-diffusable agents (for example enzymes) are transported predominantly into the liver and spleen[37,95]. Entrapped small molecules such as 5-fluorouracil[62] and penicillin[70] diffuse from the carrier into the blood while others (for example actinomycin D[70], colchicine[69]) are retained and follow the carrier to its destination. We have found that retention of daunomycin and melphalan by liposomes is greatly augmented when the two drugs are bound to DNA and polyglutamic acid respectively (G. Gregoriadis, S. C. Scott and P. N. Davisson, unpublished work). Rates of liposome clearance from the site of injection as well as penetration of target areas can easily be varied by controlling liposomal size, surface charge and lipid composition. Thus, intravenously injected small liposomes circulate in the blood for longer periods[69] and, because of their size, can reach malignant tissues[48]. Similar liposomes given intramuscularly enter the lymphatic nodes draining the injected tissue[96]. On the other hand, liposomes composed of phospholipids resistant to gut phospholipases at the body temperature are capable of transporting insulin from the gut into the blood circulation and of reducing blood glucose levels in normal and diabetic rats[51]. Indeed a considerable body of experimental evidence, discussed in detail elsewhere[35], has now made possible a realistic appraisal of the carrier potential of liposomes in the treatment of diseases as diverse as cancer, diabetes and lysosomal storage diseases and also in preventive medicine (for example as immunological adjuvants).

Although manipulation of the physical parameters of liposomes can modify to some extent the pattern of their tissue distribution[35], a method for targetting liposomes would vastly expand their potential. A few attempts for improving target selectivity have already been made: after *in vitro* exposure of phagocytes from *Mustelus canis* and a Tay Sachs disease patient to liposomes coated with aggregated isologous immunoglobulin, there was considerable improvement in the cellular uptake of the liposome-associated horseradish peroxidase[46] and hexosaminidase A (ref. 43) respectively. It was assumed that association of liposomes and their enzyme contents with phagocytes was mediated through the Fc portion of the extraliposomal immunoglobulins. Other workers[97] found that incorporation into the liposome structure of a sialoglycoprotein extracted from the erythrocyte membrane, could facilitate *in vitro* binding of such liposomes to erythrocytes in the presence of lectins. Our studies have shown that homing of liposomes is also possible *in vivo*. Thus, desialylated fetuin (which, as mentioned earlier, exhibits affinity for the hepatic parenchymal cells) incorporated on to the liposomal surface was found[50] to mediate uptake of the liposome-associated bleomycin by the relevant receptors on the liver cell surface.

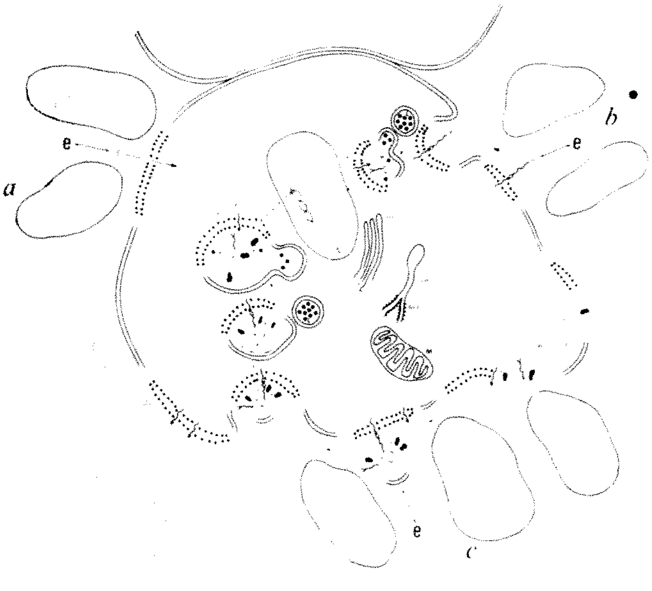

Fig. 1 Interaction of carrier-associated drugs with cells. A drug molecule (*) presented to a cell in its free form can enter the interior non-specifically by mechanisms such as diffusion and endocytosis[82] (*a*). Untoward reactions may occur because of the availability of the free drug to, and its interaction with, the environment (e). In *b*, interaction of the carrier–drug complex (α–α) with a carrier-recognising receptor (→) on the cell membrane induces endocytosis of the complex and its subsequent interiorisation into a lysosome where lysosomal exterases (●) liberate the drug. Other mechanisms of action of the carrier-bound drug are also possible[2]. Carrier-bound drug molecules are again available to, and can interact with, the environment but owing to the size of the complex and its affinity for the target, diffusion to non-target areas is reduced. *c*, A drug-containing monolamellar liposome recognises the target cell through a homing macromolecule (—<) inserted on the liposomal surface and which binds to the relevant cellular receptor (→). Contact with the cell is followed by endocytosis of the carrier (arrow pointing to the left) and its subsequent localisation in the lysosomes. Lysosomal esterases (phospholipases?) disrupt the bilayer and free the drug which can either act locally or, after escape from lysosomes, reach other cell compartments and interact with molecular targets (for example interaction of actinomycin D with DNA in the nucleus). Alternatively the liposome, upon fusion with the cell membrane (arrow pointing to the right), introduces its water-soluble contents into the cytoplasm and makes them available to the remainder of the cell. Lipid-soluble agents (▮) carried by the lipid bilayer of the liposome are transferred after fusion into the framework of the cell membrane. Liposome associated drugs, being surrounded by the lamellae, are not available to the environment. N, nucleus; GA, Golgi apparatus; SER and RER, smooth and rough endoplasmic reticulum; M, mitochondrion; L, lysosome.

The possibility of a more general method for the selective uptake of liposomes by cells is under investigation in my laboratory. Our approach[50] is based on the anchoring of native anti-target IgG immunoglobulins, through their Fc portions, into the liposomal lipid phase. The Fab moiety seems to extend extraliposomally (G. Gregoriadis and E. D. Neerunjun, unpublished work), and is capable of recognising, and associating with, the respective antigens on the target cell surface. Before trying to raise antibodies to cell specific antigens we experimented with the possibility of using whole cells for antibody production. It was hoped that the presence of cell-specific strong antigenic determinants on the cell surface would produce antisera rich in antibodies to such antigens and therefore reasonably specific for the parent cells. Indeed, after exposure of HeLa cells and human skin fibroblasts to liposomes containing ^{111}In-labelled bleomycin and bearing on their surface ^{125}I-labelled IgG raised against whole HeLa cells, the uptake of both radioactivities by HeLa cells was 25 times greater than that with fibroblasts[50]. On the other hand, after exposure of fibroblasts to the same liposomes now bearing anti-fibroblast-labelled IgG, uptake of the two radioactivities was five-fold greater than with HeLa cells[50]. As both cell types exhibited similar rates of uptake of bleomycin-containing liposomes coated with nonspecific IgG, it was concluded that cell-specific IgG on the liposomal surface was mediating selective uptake of the carrier and its drug contents. In practical terms, this approach has not only made the need for production of IgG against purified cell-specific antigen doubtful, it also suggests that purification of anti-target IgG from the multitude of other IgG molecules in the serum may be unnecessary; assuming a random distribution of the IgG molecules within the liposomal population, each individual liposome would be expected to possess on its surface some of the target-specific IgG. These specific molecules would then mediate the interaction of the associated carrier and the target. With regard to allergic reactions to the IgG used for homing, it is conceivable that insertion of the Fc portion of the immunoglobulin molecule into the lipid bilayer (which still allows the Fab portion to recognise its antigen) could mask this strongly antigenic site of the protein itself and thus prevent its interaction in the circulation with the respective anti-IgG (Fc) antibodies. Such avoidance of antigen–antibody interaction has been already observed for liposome-associated diphtheria toxoid[58], asparaginase[44] and insulin[54].

Effective direction of liposomes to target areas in the body will depend crucially on the circumvention of at least two obstacles. First, and when the target is not the hepatic parenchymal cells or in the confines of the reticuloendothelial system, for which conventional liposomes show preference, it will be necessary to reduce hepatic and splenic interference. Modest attempts[98,99] have shown that this is possible. Second, the size and composition of liposomes must be manipulated in a way that will allow passage of the carrier through a variety of anatomical barriers. This too is under investigation[48] but as there is a minimum (12 nm) in the radius of liposomes, there will be limitations. Other mechanisms, however (for example the use of leukocytes[43]), by which liposomal contents can be somehow transported into restricted areas cannot be excluded.

Provided that such difficulties can be overcome, it would be possible for specialised liposomes to seek and reach their cellular target site, become associated with it and deliver their contents. Two mechanisms of cell–liposome association are currently recognised[35], namely endocytosis[100–102] and fusion[45,73,103–105] (Fig. 1*c*). Direct as well as circumstantial evidence[35] suggests that the former mechanism occurs both *in vitro*[106,38] and *in vivo*[101,102,107] with a variety of actively or not so actively endocytosing cells. Because of this process, liposome-associated agents end up in the lysosomes where they are liberated and assuming such agents retain their activity in the lysosomal milieu, they can then act locally[106,38,108]. Alternatively, and when the physical properties of the agents allow it, these can escape by diffusion and reach other cell regions[61]. There is now considerable evidence[73,103–105,109] to suggest that under certain conditions *in vitro* the liposomal bilayers can, in addition to being endocytosed, fuse with the cell membrane (Fig 1*c*). This can either mediate incorporation of lipid soluble substances (located within the lipid phase of liposomes) into the cell membrane[110] or introduction of water soluble substances (entrapped in the aqueous space) in the cytoplasm[73].

Selective association of liposomes with cells could bring agents with their own selectivity for action near intracellular molecular targets. For instance actinomycin D or daunomycin originating from lysosomes (endocytosis) or from the cytoplasm (fusion), could enter the nucleus, bind to DNA and inhibit DNA directed RNA synthesis[61]. Similarly, agents in the vicinity of mitochondria or other organelles could, because of a specific affinity, bind to respective components and exert their effect. It thus appears that targetting of drugs with liposomes could proceed in two steps. In the first, the carrier would recognise the general area of action and subsequently enter it. Following their liberation from liposomes, drugs could then proceed for a finer target recognition at the molecular level (Fig. 1).

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articles

Additional results on palaeomagnetic stratigraphy of the Koobi Fora Formation, east of Lake Turkana (Lake Rudolf), Kenya

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The magnetostratigraphy of the hominid-bearing sediments exposed east of Lake Turkana has been strengthened by new palaeomagnetic results. Ages obtained from several tuffs by the ^{40}Ar/^{39}Ar method suggest an approximate match between the observed magnetozones and the geomagnetic polarity time scale; however, the palaeomagnetic results are also compatible with a younger chronology suggested by conventional K–Ar dating of the KBS Tuff.

BROCK and Isaac[1] have reported palaeomagnetic polarity determinations from the Koobi Fora Formation, exposed on the north-east side of Lake Turkana (formerly named Lake Rudolf), Kenya (Fig. 1). Because these Pliocene and Pleistocene deposits have yielded rich assemblages of fossil vertebrates, including the remains of very early humans, the ages of these beds have received considerable attention[2–4]. The Koobi Fora beds have also yielded a large collection of stone artefacts representing at least two toolmaking industries[5,6]. The palaeomagnetic study complemented a framework of radiometric ages determined from five tuffs interbedded within the Koobi Fora formation[7]. After Brock and Isaac's results were published, one of the tuffs, the KBS, was redated in another laboratory using the conventional K–Ar method, and a younger age was reported[8]. We have now obtained additional palaeomagnetic results which warrant a reevaluation of the magnetic stratigraphy.

The palaeomagnetic study of Brock and Isaac included 247 oriented specimens collected from four stratigraphic sections within the Koobi Fora Formation. The sedimentary deposits consist of fluvial sandstones and siltstones interbedded with lacustrine mudstones that formed near the eastern shore of ancient Lake Turkana. The palaeomagnetic results were tied to local, discontinuous outcrops of tuffs, each of which has been tentatively correlated with one of five ash beds that are believed to be widespread marker units within the Koobi Fora area. Listed from oldest to youngest, these tuff horizons have been named: Suregei, Tulu Bor, KBS, Koobi Fora and Chari. The proposed correlation of tuffs should be considered as a working hypothesis subject to revision. Further work is now in progress and it is evident that some amendments to the published correlations[9–12] are called for (I. Findlater, J. M. Harris, T. White and R. E. F. Leakey, personal communication.).

A consistent sequence of polarities was established from the four stratigraphic columns, and using the previously published age of 2.6±0.26 Myr for the KBS Tuff[13] as a fixed point, this sequence was matched with parts of the geomagnetic polarity time scale. In this interpretation part of the lower member of the Koobi Fora Formation, bracketed by the KBS and Tulu Bor Tuffs, was assigned an age of 2.6–3.2 Myr, while the upper member was assigned an age between 1.2 and 1.8 Myr. As reversed rocks were not found between the Koobi Fora Tuff and the KBS Tuff, the interpretation required that sediment representing 600,000 yr of the early Matuyama epoch had been removed from the section, possibly by uplift and erosion after deposition of the KBS Tuff. The subsequent publication[7] of ages for the other tuffs, namely, Tulu Bor (3.18±0.09 Myr), Koobi Fora (1.57 Myr) and Chari (1.22±0.01 Myr) provided strong support for the palaeomagnetic interpretation.

The basis of the initial polarity interpretation was the KBS Tuff, the age of which is now disputed. An age of 2.6±0.26 Myr was initially assigned to the KBS Tuff on the basis of apparent age spectra determined by the ^{40}Ar/^{39}Ar method, which entails step-wise heating of specimens. It was thought that the conventional K–Ar method could not be applied successfully to the Koobi Fora tuffs because the ash had lost some argon, due possibly to widespread hydrothermal events[14]. Conventional K–Ar

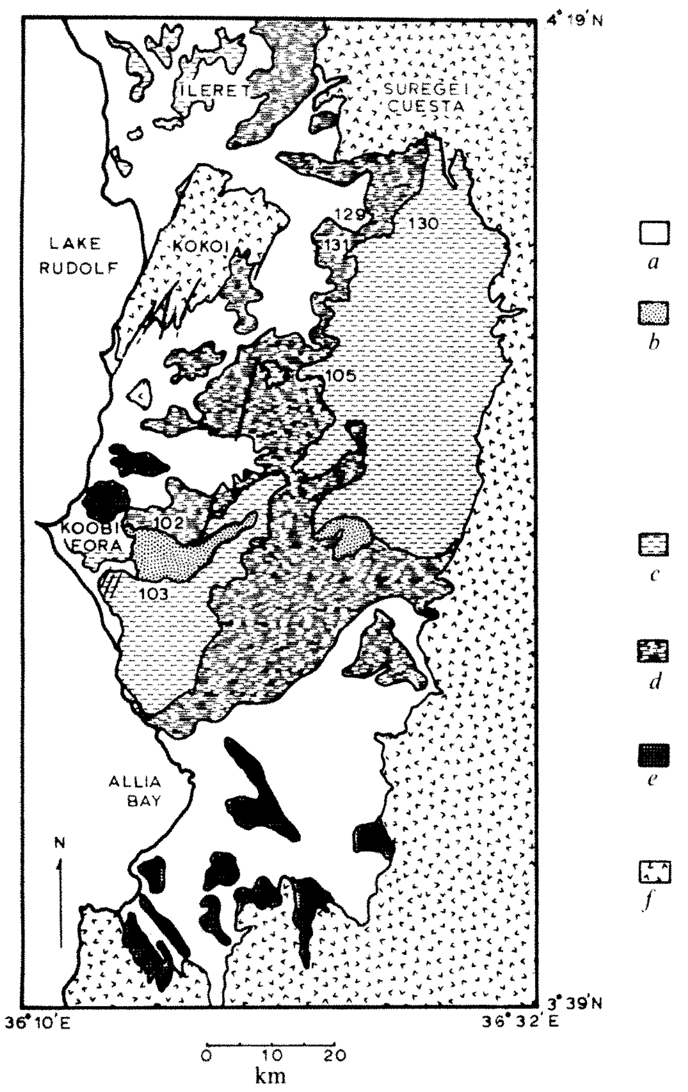


Fig. 1 Geological map of the Koobi Fora region in northern Kenya, showing palaeomagnetic sampling area (after Vondra and Bowen (ref. 11). *a*, Alluvium, beach sand; *b*, Galana Boi beds. *c*–*d* Koobi Fora Formation. *c*, Upper member; *d*, lower member; *e*, Koobi Algi Formation; *f*, volcanic rocks undifferentiated.

dating has now yielded significantly younger ages[8]. Glass and sanidine separates taken from the same sample have remarkably similar ages; nine specimens from areas 105 and 10 gave an average age of 1.60±0.05 Myr and six specimens from area 131 gave an average age of 1.82±0.04 Myr. These results combined with trace element analysis indicated that the tuffs exposed in areas 105 and 131 might be from two eruptions separated in time by 200,000 yr. The high precision and concordancy of these dates determined from two materials with different crystal structures provide strong evidence that the tuff has not lost radiogenic argon. Additional ^{40}Ar/^{39}Ar measurements[15] and fission track age determinations[16], however, provide evidence in favour of the earlier date for the KBS Tuff. Thus the chronology of the Koobi Fora Formation remains open to discussion, and alternate interpretations of the magnetostratigraphy, enhanced by 800 additional samples, are presented here.

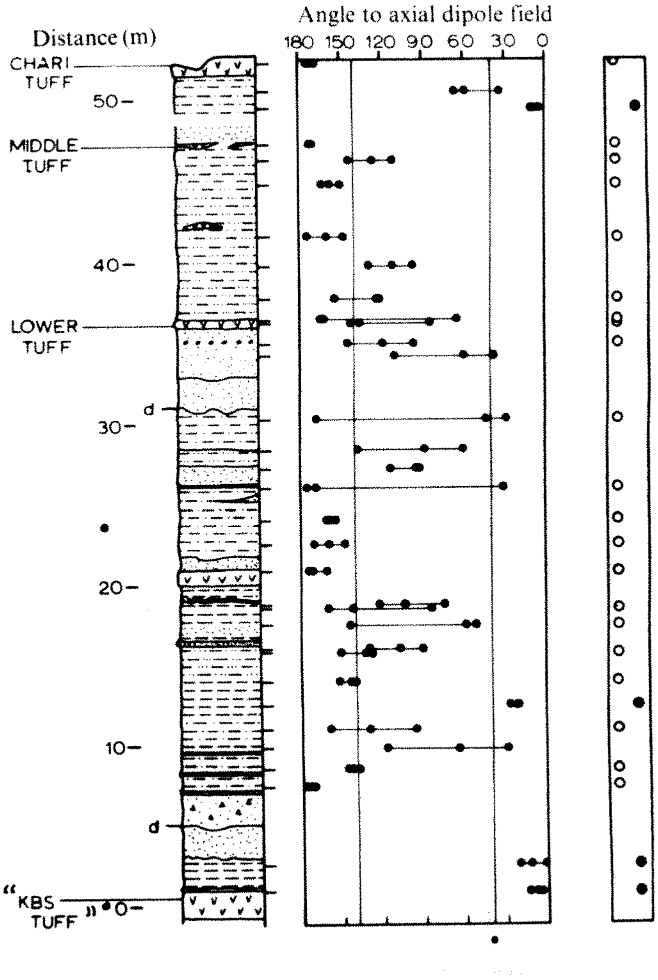
Additional palaeomagnetic results

We have determined magnetic polarities of six new stratigraphic sections, spanning the following stratigraphic intervals (Figs 1 and 4): (1) area 131: 'Tulu Bor'–'KBS Tuff'; (2) area 105 and 116: 'Tulu Bor Tuff'–KBS Tuff (type section); (3) Ileret: 'KBS Tuff'–Chari Tuff (type section); (4) area 129 and 15: Suregei Tuff–Tulu Bor Tuff (type section); (5) area 102: 'Suregei Tuff'–Koobi Fora Tuff; (6) area 103: 30-m column just below the Koobi Fora Tuff (type section).

Because the identities of tuffs in localities other than the type areas are hypothetical, the above list indicates whether or not the palaeomagnetic column is attached to a type section. In the remainder of this paper, tuffs which have not been traced back to the type section with certainty are flagged with quotation marks.

The sampling technique has been described by Brock and Isaac[1]: The magnetic polarity of each horizon was determined from three oriented cubes (2 cm × 2 cm × 1.5 cm) with a 5 c.p.s. flux-gate spinner magnetometer. One sample from each horizon was progressively demagnetised with an alternating-field (AF) up to 300 Oe, then the optimum field was selected as that which minimised the change in magnetic direction between successive demagnetisation steps. The remaining samples were demagnetised at the optimum field determined from the pilot sample. Typical optimum fields of these samples ranged between 100 and 200 Oe, with the remaining magnetisation averaging 10–25% of the initial natural magnetisation. Almost all of the specimens had acquired

Fig. 2 Palaeomagnetic results from the Ileret section. The angular difference between the cleaned palaeomagnetic direction and the normal axial dipole field is plotted for each sample. On the right the polarity of each horizon has been interpreted as normal (dot) reversed (open circle), or indeterminate (no symbol) from analysis of three samples. Disconformities are indicated by d. The tuff correlated as the 'KBS' is the uppermost of a pair of tuffs separated by several metres of sediment. This same upper tuff was dated by Curtis *et al.*[8] at 1.60±0.05 Myr.



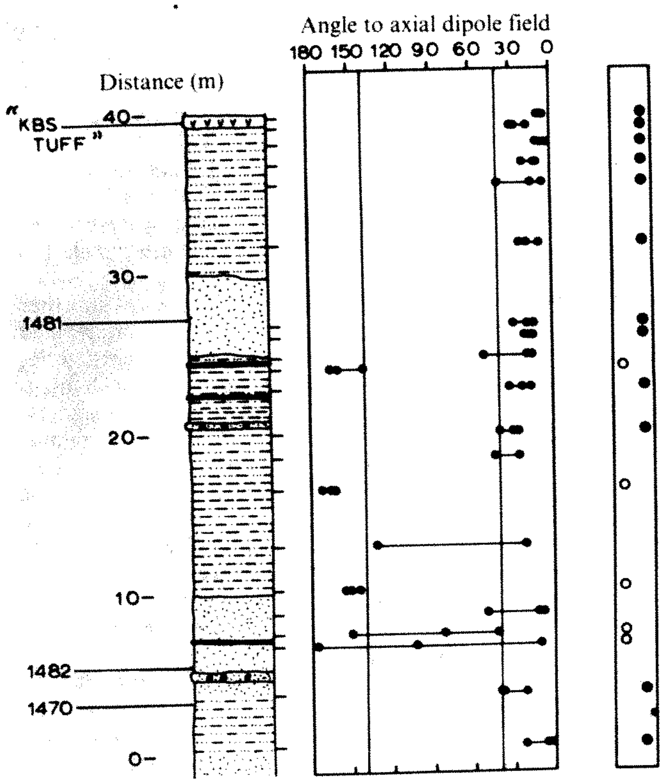


Fig. 3 Palaeomagnetic results from the lower member of the Koobi Fora Formation in area 131. Stratigraphic positions of hominid sites ER-1470, ER-1482, and ER-1481 are indicated[3]. The Tulu Bor Tuff is believed to be located approximately 6 m below the base of this section. The tuff correlated as the 'KBS Tuff' in this column was dated by Curtis *et al.*[8] at 1.82±0.04 Myr. The possibility cannot be eliminated that this tuff is the lateral equivalent of a minor tuff that occurs below the type KBS Tuff in area 105.

viscous magnetisation parallel to the normal axial dipole field, and this component was effectively removed by AF demagnetisation of 50–100 Oe.

In the treatment of the preliminary columns, Brock and Isaac[1] defined normal and reversed magnetic directions as those which deviated less than 50° from the axial dipole ($D=0°$, $180°$; $I=\pm 8°$). Directions lying outside these 50° cones were regarded as intermediate. In a few cases the cleaning procedure produced erratic changes of direction between successive steps, so these samples were classified as giving no clear result. In order to give a better representation of the range of dispersion within horizons we have modified this analysis by computing for each sample the angle between the cleaned palaeomagnetic direction and the normal axial dipole field ($D=0°$, $I=8°$). Because all of the sampling localities are located within a 30-km radius, magnetic directions rather than virtual geomagnetic poles (VGP) were analysed. As examples, the palaeomagnetic results from columns in areas 131 and Ileret are shown in Figs 2 and 3.

We have defined the polarity of each specimen using cones of confidence centred about the normal and reversed axial dipole directions. Given a period of 10^5 years or more, the time-averaged direction of the Earth's field at a given site is very close to the axial dipole direction, although at a given instant changes in the non-dipole field may displace the field vector away from the axial direction. At the latitude of Koobi Fora (4 °N) the angular standard deviation is 18°, based on a model which assumes non-dipole dispersion similar to that of the present field[17]. Palaeomagnetic evidence from Tertiary lavas indicates that this value of the standard deviation is in accord with ancient field behaviour[18,19]. We have used an angle (40°) slightly larger than two standard deviations as the radius of the 95% confidence cone centred on the axial dipole in order to define the polarities as follows: (1) If the angle to the axial dipole lies between 0° and 40° the specimen has normal polarity. (2) Between 140° and 180°, the specimen has reversed polarity. (3) Between 40° and 140° the specimen has intermediate polarity.

Even after AF cleaning, some horizons gave three widely scattered directions, and in extreme cases the horizons contained normal and reversed polarities. This inconsistency indicated that some samples had acquired secondary remanence which was not completely removed by AF demagnetisation. Therefore the polarity of each horizon was interpreted from three specimens according to the following rules: (1) If one or more specimens had reversed directions, the horizon was assigned reversed polarity. (2) If all three specimens had normal directions, the horizon was assigned normal polarity. (3) If a horizon had one or more intermediate specimens and no reversed specimens, the results were rejected and the horizon's polarity was said to be indeterminate. Intermediate directions may record true palaeofield directions during polarity transitions or excursions, or they may be due to the superposition of primary and secondary magnetic components with opposite polarities. Because of this ambiguity, intermediate directions were ignored in the interpretation of the magnetic stratigraphy.

This scheme deliberately favours reversed directions. Our reason for adopting it is that many Koobi Fora specimens have acquired an overprint of stable secondary remanence with normal polarity. This normally polarised secondary remanence may have been acquired by two processes; it may have originated as viscous remanence due to recent exposure in the Earth's field or it may have originated as chemical remanence by oxidation of magnetite during weathering. Because the Earth's field has been normally polarised essentially all of the time since 700,000 BP we would expect the viscous magnetisation to be normal. Recent weathering of outcrops may cause chemical changes that also impart normally polarised chemical remanence. For these reasons, reversed magnetisation is less likely to represent overprinting due to viscous magnetisation or weathering and therefore is more reliable for purposes of palaeomagnetic stratigraphy. Some specimens may have acquired post-depositional chemical remanence during periods of reversed polarity; however, at Koobi Fora long intervals of normally polarised rocks contain only a few intermediate and reversed specimens; they tend to be entirely normal. On the contrary, horizons with widely scattered directions are confined almost entirely to beds that have reversed polarity. This indicates that for the most part secondary remanence is normally polarised. In short, reversed magnetisation indicates that the rocks formed when the field was reversed whereas the converse is not always true for normal magnetisation. Good examples of the relations defined above can be seen in the Ileret and area 131 sections (Figs 2 and 3). It was for the above reasons that reversed directions were given more weight than normal directions in the interpretation of the magnetic stratigraphy.

Figure 4 summarises the magnetic stratigraphy of the Koobi Fora Formation, including the preliminary results[1] in columns marked with arrows. Two incomplete sections, Ileret 6A and area 103, which were presented by Brock and Isaac, have been superseded. Although some inconsistencies are apparent between the sections, a coherent pattern may be seen which provides a basis for making correlations with the geomagnetic polarity time scale. Besides extending the magnetostratigraphy down to the Suregei Tuff, the new results have led to some revisions of the preliminary magnetozones. For example, in area 105 between the 'Tulu Bor' and the type KBS Tuffs, Brock and Isaac found evidence for two reversed zones separated by a short

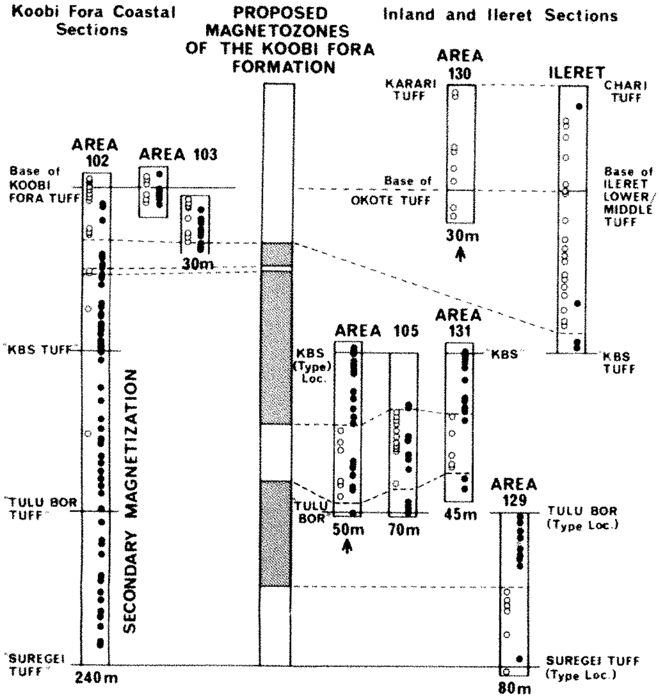


Fig. 4 Summary of palaeomagnetic stratigraphy of the Koobi Fora Formation. The stratigraphic sections are correlated by several key tuffs, placed on an arbitrarily spaced grid. The actual thicknesses are shown at the bottom of each stratigraphic section. Because the correlations of tuffs outside the type localities are provisional these correlations are flagged with quotation marks. Normal polarities (dot) and reversed polarities (open circle) define magnetozones shown in the generalised column. Columns previously reported by Brock and Isaac[1] are marked with an arrow.

normal zone. Resampling of the same interval in areas 105 and 131 showed the presence of sporadic normal horizons within the thick reversed zone. In keeping with our assumption that reversed magnetic directions are more reliable than normal ones, we have disregarded those isolated normal horizons in constructing the generalised magnetic column in Fig. 4. Therefore, we now regard the reversed horizons below the KBS Tuff as part of one magnetozone.

The inconsistencies serve as a warning that palaeomagnetic dating, like K–Ar dating, is subject to errors due to geochemical processes. For example, the lower portion of the Area 102 column (Fig. 4) is normally polarised, whereas sections of overlapping age in areas 129, 131 and 105 have several bands of reversed polarity. In area 102 the oldest sediments consist of brittle, grey claystones deposited in deep water within a rapidly subsiding graben. In contrast the equivalent deposits exposed in areas 105 and 131 are composed of fluvial and shallow lacustrine sediments. Apparently, the original palaeomagnetic signal carried by the area 102 clays was erased, perhaps by chemical alteration of the detrital magnetic minerals during burial. In recent time the clays may have acquired stable chemical magnetisation of normal polarity. Palaeomagnetic results from the lower portion of the area 102 column were therefore largely discounted. As a general rule, the least reliable palaeomagnetic columns of the Koobi Fora formation were located in hard lacustrine clays exposed near the present shoreline. The most consistent results were obtained from deltaic and shallow lacustrine sediments exposed near the eastern margin of the Koobi Fora beds.

In all columns, however, mixed polarities and scattered magnetic directions were most apparent in poorly consolidated sandy beds where the mean grain size exceeded 50 μm. For example, the reversed magnetozone from area 103 is interspersed with isolated normal horizons and many of the reversed horizons include one or two specimens with normal or intermediate polarity. Most of these unreliable results were determined from friable, coarse-grained sediments. In the area 102 column, scattered magnetic directions occur between the 'KBS' and 'Tulu Bor Tuffs'. This part of the column largely consists of sandstone. It is possible that the entire column from area 102 is affected by remagnetisation, and that in Fig. 4 we have overestimated the true thickness of the normal magnetozone which lies above and includes the 'KBS'.

Post-depositional normal polarity may be present in other areas, so the magnetic stratigraphy should be interpreted cautiously, especially where short normal magnetozones are present. We have attempted to determine which magnetozones correspond to real changes in geomagnetic polarity by repetitive sampling of palaeomagnetic columns throughout the Koobi Fora Formation.

There is an element of ambiguity in palaeomagnetic dating because it involves recognition of a pattern composed of only two elements; the identify of a particular time interval is determined from the relative thicknesses of adjacent polarity zones. Where deposition has been steady and where the magnetic recording process has been of good fidelity, palaeomagnetic ages can be assigned with the known reversal time scale. Hiatuses and changes in the deposition rate, however, which are common in the fluvial and lacustrine facies of the Koobi Fora Formation, have distorted the polarity sequence. Additional age controls provided by the fossil record and by K–Ar dating are needed to provide an approximate match with the known reversal time scale. The palaeomagnetism can then be used to define the ages of some of the key strata.

Interpretation of magnetic stratigraphy

We have constructed a generalised stratigraphic column which shows the relative positions of the tuffs and the observed magnetozones of the Koobi Fora Formation (Fig. 5). With control provided by the framework of radiometric ages of the tuffs, the magnetozones are correlated with the geomagnetic polarity timescale[20–22]. As the age of the KBS Tuff remains under discussion we propose two possible interpretations of the Koobi Fora magnetic stratigraphy. Interpretation 1, proposed originally by Brock and Isaac[1], is based on a KBS age of 2.6 Myr obtained by the ^{40}Ar/^{39}Ar step-heating method. Interpretation 2 is an alternative which is in accord with the KBS complex age of 1.60–1.82 Myr obtained by Curtis and others[8] who used the conventional K–Ar technique.

In both interpretations the age of the upper member, which lies above the KBS Tuff, is between 1.2 and 1.8 Myr; however, the age of the top boundary of the lower member differs by 1 Myr. If interpretation 1 is accepted, the first reversed magnetozone below the KBS Tuff correlates best with the combined Mammoth and Kaena events, 2.8–3.1 Myr. Accordingly, the reversed interval below the Tulu Bor Tuff correlates with the end of the Gilbert epoch, 3.3–3.7 Myr. Because a substantial interval of reversed polarity is missing between the KBS Tuff horizon and the 1.61 Myr polarity boundary, this interpretation requires that sediments of early Matuyama age, 1.8–2.4 Myr, are not present in the Koobi Fora area. It has been proposed by Brock and Isaac that this time gap is due to uplift and subsequent erosion. Indeed, a disconformity was mapped in area 105, area 103, and Ileret just above the 'KBS Tuff'[5]. Whether the corresponding hiatus is as long as the required 600,000 years is uncertain, however, and this uncertainty remains a major weakness of the chronology.

Interpretation 2, which is consistent with an age of 1.6–1.8 Myr for the KBS Tuff complex, assigns an age of 1.8–2.4 Myr to the first reversed magnetozone below the KBS Tuff. Reversed horizons below the Tulu Bor Tuff are in accord with the combined Mammoth and Kaena events

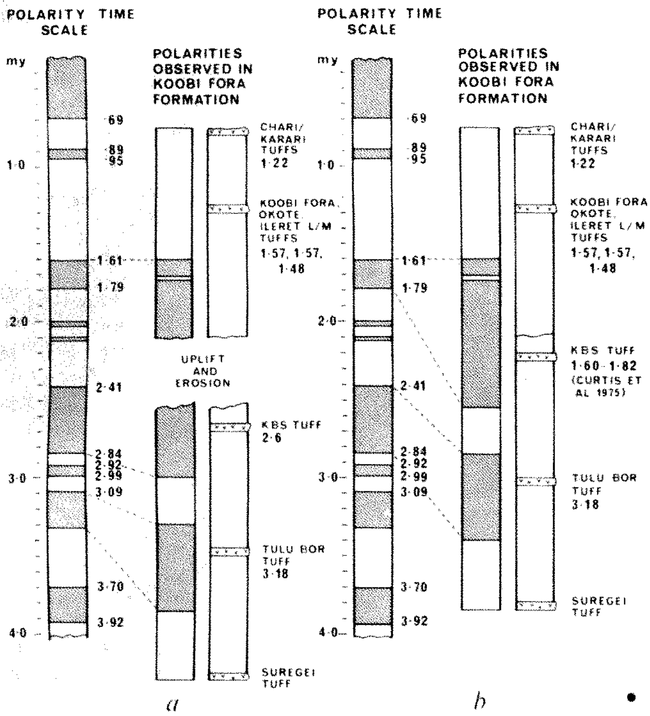


Fig. 5 Two possible correlations of the magnetozones observed in the Koobi Fora Formation with the geomagnetic polarity time scale. *a*, Interpretation 1 is based on ^{40}Ar/^{39} dating of the KBS Tuff (2.6 Myr) by Fitch and others[7]. *b*, Interpretation 2 is based on conventional K–Ar dating of the KBS Tuff, which gave two ages, 1.60 Myr in area 105 and 1.82 Myr in area 131[8]. All other ages were determined by the ^{40}Ar/^{39} Ar method.

(2.8–3.1 Myr). In contrast with the original chronology, interpretation 2 reduces the age of the reversed magnetozone between the Tulu Bor and KBS Tuff by 0.7–1.0 Myr and increases its duration from 0.3 to 0.6 Myr. Interpretation 2 also suggests that the Tulu Bor Tuff is younger than 2.8 Myr, contradicting the tuff's radiometric age of 3.18±0.09 Myr, and this must be accepted as a major weakness of the interpretation.

Fossil mammal assemblages offer another means of calibrating the polarity sequence at Koobi Fora against other well-dated localities in East Africa. Palaeontologic, palaeomagnetic, and radiometric ages have been obtained from the Pliocene and Pleistocene Shungura Formation, located along the Omo River in southern Ethiopia[23]. The occurrence of the fossil pig *Mesochoerus limnetes* in both the Koobi Fora and Shungura Formations helps in the correlation of the magnetozones between the two areas. Cooke and Maglio[24] reported that *Mesochoerus limnetes* from below the KBS Tuff at East Turkana correlates best with Shungura Member F which has a K–Ar age of approximately 2.0 Myr (ref. 23). The biostratigraphic range of a single species can vary between regions depending on palaeoecological conditions, so the above correlation must be weighed against other lines of evidence. Recent collecting in these areas has provided a larger sample of the fossil fauna which is now being analysed (J. M. Harris, personal communication). Note that neither geophysical methods nor biostratigraphy yet provide an unequivocal chronology for the Koobi Fora Formation.

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Note added in proof: Continuing studies show partial error in tuff correlations (Fig. 4): (1) Identities of tuffs are unknown in the lower portion of area 102 column, so we can no longer assume that this section was overprinted with normal polarity. (2) A stratigraphic gap could exist between the bottom of column 105 and the top of column 129 because area 105 basal tuff cannot be firmly correlated with the type Tulu Bor Tuff of area 129. 'Area 129' column is a composite: the upper portion was from area 129, the lower, from the adjoining area 15.

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Aggregation, migration and population mechanics

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A concept is developed for the regulation of populations by density-dependent movement, rather than by overt competition alone. Fitness is seen as maximising the reproductive advantage of a balance between migratory and congregatory behaviours. Population density is shown to be spatially, as well as temporally dynamic and a mechanism is proposed that accounts for observed spatial behaviour.

THE Malthusian concept of population is contained in the basic proposition that, with full census information, population change can be assigned to either reproduction or mortality[1]. This proposition is consequently the basis of Darwinian fitness measured in terms of reproduction and survival[2], and of population models for competition[3,4] and predation[5,6] that are primarily concerned with equilibrium conditions[7,8]. Populations are seen as persisting through time owing to the check on multiplication imposed by such familiar Malthusian[9] restraints as disease, predation and resource limitation. Key factors[10,11] direct our attention to the causes and time of death, but not to the place of death nor to its distance and direction from the place of birth. Lacking this component of movement in real space, it seems to us that populations are static[12], not dynamic. They can neither function nor evolve unless they include the controlled mobility that enables organisms to select a place to live and so survive and reproduce. This mobility we shall equate largely, but not entirely, with internal or external migration using the word in a wider sense than that familiar in ornithology.

Migration and static population concepts

Until recently migration has often been regarded as an idiosyncrasy to be included with unaccountable mortality in life tables based on census information from a restricted area. Yet migration is not synonymous with mortality. Re-examination of old census records now reveals more extensive, successful, internal migration in man than was formerly appreciated[13,14]. Among insects, many species formerly regarded as sedentary have been found to be vagile[15]. For example, *Drosophila* seem non-migratory as compared with the monarch butterfly[16], and their flight behaviour is well adapted to minimising dissemination[17,18]. Even so, their migratoriness is now known to be considerably underestimated in the Dobzhansky–Wright models that so influenced concepts of population mobility in genetics[19,20]. New evidence from bird ringing shows long distance movements in territorial species formerly considered extremely sedentary, the European blackbird, for example[21], and, since young birds rarely occupy their parents' nests, they must migrate to establish a new territory. Dispersal in small mammals has been related to population cycles[22] and the zoo syndrome has been called "frustrated dispersal"[23] in species not commonly thought of as migratory.

Segregation of migration from dispersal obscures their common population function. In birds, fish and especially in insects, whose complex life-histories enable the various aspects of migration to be analysed in most detail[24,25], much is now known about ontogenetic[25], physiological[26] and behavioural[27] processes that initiate movement and about its evolutionary origins[28] and ecological function[29], as well as about the more striking, but in this context less relevant, orientation[30,31] and navigation[32] behaviour. Gene migration models[33-35], like population models[36], have not yet made due allowance for the sophisticated migration control mechanisms in which the experience of parents and grand-parents[37], as well as population density[38], affect the migrant status of the individual. These are most clearly evident in the labile[39] migratory polymorphisms, remote from genetic heterogeneity, in clonal populations of aphids[40]. As a result, little light has been shed on the role of migration in the dynamics, and hence in the fundamental concept, of population.

Concepts of population are not consistent[11]. A lack of rigour in distinguishing total population from population density can imply uniform distribution of density in space, whereas the actual spatial distribution of few organisms is known[42]. Again, key factors differ for the same species in different places and affect different stages in the life history. This makes models for the whole species population incomprehensible. If, however, a model is considered to represent a separate element of the population, equivalent to the mean density at a certain place, then growth rate can be equated with unity, as required by basic theory[9,43], only if there is no population flow between such places.

But we doubt that populations are often like that. All populations are spatially fluid in some measure because movement is a fundamental biological response to adversity. Nor is population density distributed uniformly in space. A valid concept of population must encompass both the more settled species, like the red grouse say[44], to which the current models appear to be most suited, and the highly volatile species, like aphids, for which local stability in time seems almost irrelevant because of the persistent change of place.

A spatial concept for population and some evidence

The classical model for the whole population of a species changing only through time was the phylogenetic tree. If we treat the anatomy of a real population as being in three dimensions, latitude × longitude through time, we visualise its internal structure to be reticulate, not unlike the fern stele in Fig. 1 in which density changes constantly through time and space. So, spatial sections through this anatomical model at successive time intervals differ slightly, or markedly, depending upon the time scale. (This has much in common with MacArthur's[45] r–K continuum, and with Southwood's[46] τ/H ratio.)

After monitoring the changing distribution of density of hundreds of species of Lepidoptera throughout Great Britain for almost a decade[47], we can show that growth cycles in different local population elements are normally asynchronous, as in Ford's marsh fritillaries[48], combining with movement to change the pattern of density in space like serial sections through the fern stele model, even when overall abundance remains relatively constant (Fig. 2). The moths we have sampled range from known migrants to species considered localised, but density distribution is always changing. The time-scale is, of course, short compared with vertebrates. Our conceptual model predicts that once a density element has been defined in space, its life-span is limited. Our field samples confirm this,

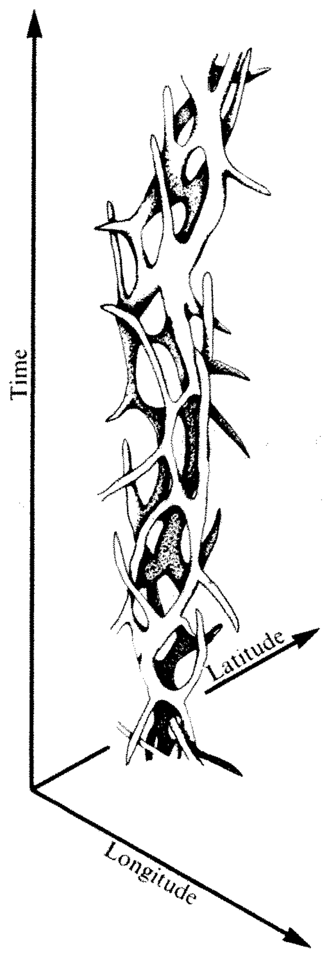

Fig. 1 Conceptual model for population anatomy based on Rostowzew's[75] drawing of the stelar structure of the adder's tongue fern. The stele represents the space-time reticulum created by the distribution of an arbitrary density level through time.

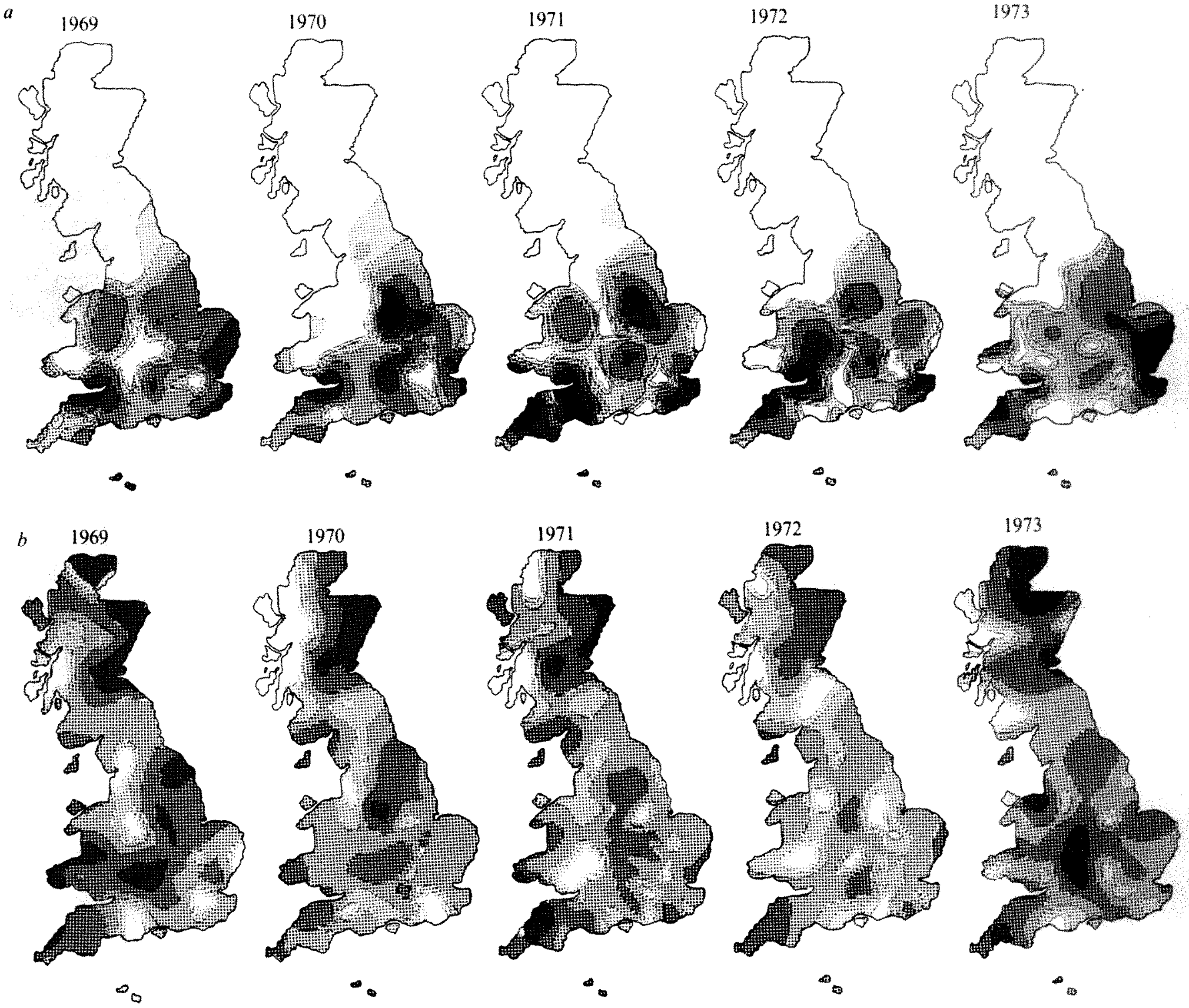

Fig. 2 Measured annual (1969–73) adult population density distribution of two moth species showing the changing patterns typical of the 623 species sampled by the Rothamsted Insect Survey[17]. Each map is based on 62–88 sample points; there were inadequately distributed in Scotland, hence the poor definition there in (*b*). Density layers 0, 1–2, 3–9, 10–31, 32–99, 100–315, 316–999, individuals per sample unit. *a*, *Agrochola lychnidis* (Denis and Schiff.) a marginal species near its northern limit. *b*, *Xanthorhoe fluctuata* (L.) has a very stable total population. The maps are visualised as serial sections through the conceptual model in Fig. 1. In both instances the appearance and disappearance of 'holes' in the population is clearly visible so that all parts of the population are essentially 'marginal'.

and hence that the use of spatial averages, in fitness say[35], makes questionable assumptions about the nature of spatial distribution, for density can diminish locally to create what are usually regarded as marginal conditions[49,50] even in the heart of a population (Fig. 2).

The individual's dilemma and the population consequence

In an environment changing through time and space, the most probable strategy for a new individual to adopt to survive and reproduce is not necessarily to stay to compete with its parents or congeners[51] but may be to go elsewhere, to find an empty environmental hole to inhabit. Its ability to do this depends on its malleable migrant status. In a doomed habitat, one offspring capable of migrating at the right time may well have more survival value than a litter of more sedentary morphs[40]. Thus the migrant status of offspring is an essential component of fitness controlling the production of grandchildren, which we take to be the relevant criterion. This point was originally made by Grinnel[52] but he assumed population centres to remain static and, like Gadgil[53] and others[35], regarded migration as compulsive for a genetically fixed proportion of the population. MacArthur[54] argued, legitimately, that such migration would be from an equilibrium population to a less hospitable place and involve a loss of fitness. Much environmental hostility which results in death or dispersal is transient, however, especially that due to biological restraints such as predation and disease or the loss of nesting sites or food supply. This creates vacant holes in the environment which subsequently recover their hospitality and are re-occupied by searching migrants so that population centres are mobile when seen at the appropriate time scale. These more fluid populations make MacArthur's argument unconvincing and, from the individual viewpoint, offer alternative courses of action. It was to an apparently social option that Hamilton[55] addressed his attention which he so graphically illustrated by his selfish frogs. The environmental menace was a predator and the choice considered was where to go within the herd so as "not to be nearest the snake". There is an additional option which Hamilton precluded, to abandon the herd and find a pond

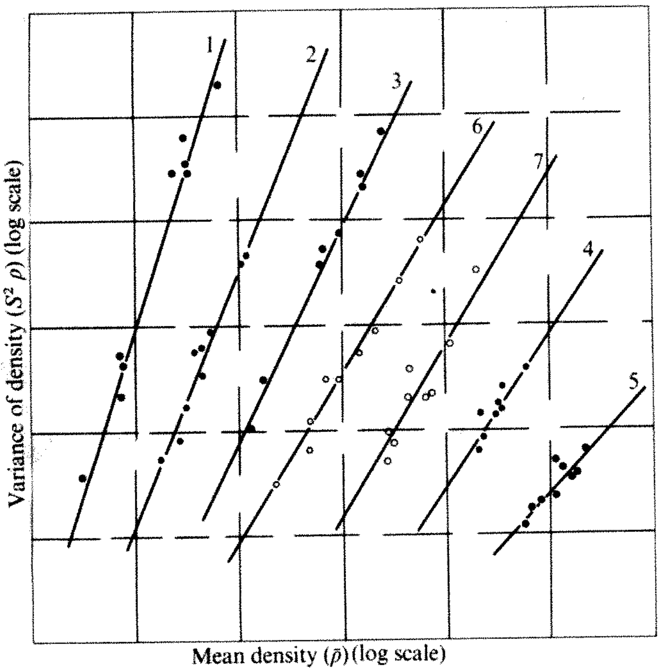

Fig. 3 The relation between variance of spatial disposition and annual mean density for five moth species from the same source as in Fig. 2, and two simulated quasi-stable populations. Each point is one years sample, equivalent to a map in Fig. 2. The fitted regression is $\log S_\rho r^2 = a + b \log \bar{\rho}$. The range of aggregatory response to overall population level differs widely between species but is highly consistent within species. 1, *Lycophotia varia* (Villers) ($a = 0.02$; $b = 3.02$). 2, *Xanthorhoe ferrugata* (Clerck) ($a = -0.29$; $b = 2.41$). 3, *Malacosoma neustrie* (L.) ($a = 0.94$; $b = 1.98$). 4, *Xestia xanthographa* (Denis & Schiff) ($a = 1.16$; $b = 1.48$). 5, *Epirrhoe alternata* (Müller) ($a = 1.56$; $b = 1.04$). 6, From Fig. 5*a*; heterogeneous environment ($a = 1.38$; $b = 1.65$). 7, From Fig. 5*b*; homogeneous environment ($a = 1.35$; $b = 1.66$). Axes are staggered to save space. Grid is at ×10 intervals. Symbols are (●) real samples, (○) from simulation.

without a snake. This individual choice creates recognisable spatial properties at population level which we suggest lead to population homeostasis without group selection[56,57].

A dynamic mechanism and a postulate

We would argue that all spatial dispositions can legitimately be regarded as resulting from the balance between two fundamental antithetical sets of behaviour always present between individuals. These are, repulsion behaviour, which results from the selection pressure for individuals to maximise their resources and hence to separate, and attraction behaviour, which results from the selection pressure to make the maximum use of available resources and hence to congregate wherever these resources are currently most abundant. The balance between these two conflicting behavioural tendencies operating on each individual determines its movements and hence the resulting spatial pattern of the population at any instant in time. It is the response of this balance to changing internal and external environmental conditions that, we suggest, constitutes the dynamic element in populations.

From this basis we have sought to bridge the gap between models and real data with a functional mechanism for the distributive processes in a population that would lead to the diffusion rates and spatial dispositions observed in nature.

The only hard spatial evidence we know of, that relates equally to populations of all kinds of organisms, is the statistical measure of fine-scale spatial disposition of individuals[58]. Randomness is by definition atypical of the microdistribution of living organisms and one of us has previously shown empirically[59] that the spatial variance ($S_\rho{}^2$) characteristic of species, at a particular point in the environment, is proportional to a fractional power of mean population density ($\bar{\rho}$) at that place;

$$S_\rho{}^2 = a\bar{\rho}^b, \qquad (1)$$

where b is a measure of the density dependence of aggregation. Our field monitoring now yields convincing evidence that macrodistribution, at least in Insecta, still has the same property of density-dependent aggregation when mean density relates to an area the size of Great Britain instead of a few square metres (Fig. 3). From this it is clear that, to return to the ferne stele model, successive stelar sections characterise the species spatial behaviour on a geographical as well as on a local scale.

The other relevant experimental evidence relates to rates of diffusion, or migration, of individuals from population concentrations, but for this there has yet been no generally accepted functional relation[60,61]. Biological diffusion is no more random than spatial disposition, for it is the outcome of equally

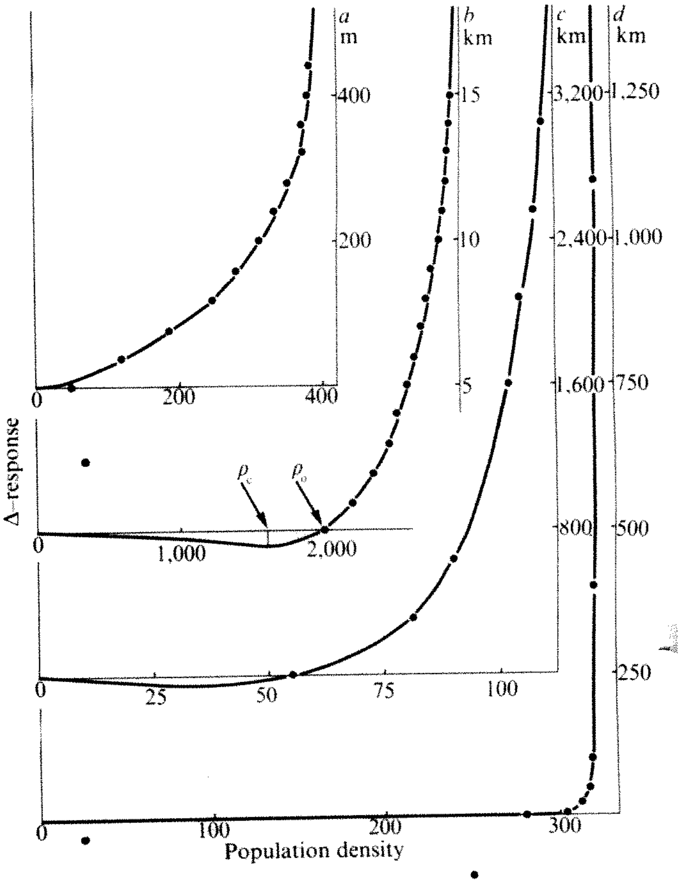

Fig. 4 Δ-functions reflecting different life styles with superimposed density × distribution data from *a*, sedentary insect, *Drosophila pseudoobscura*; fluorescent dust recoveries at attractant traps in California[20]. *b*, Social primate, man; census data from Sweden[13]. *c*, Migrant insect, monarch butterfly; wing-label recoveries in North America[16]. *d*, Territorial bird, blackbird; leg-ring recoveries in Great Britain[21].

Parameters

	ε	ρ_0	p	q
a	0.03	6	0.97	0.72
b	5.2	2000	7.3	6.7
c	11.0	54	7.2	1.1
d	7.3×10^{-22}	225	151	2.1

ρ_0 is the population density at which migratory and congregatory behaviour balance and no net movement ensues. At $\rho > \rho_0$ the population is over-dense and migratory behaviour predominates; at $\rho_c < \rho < \rho_0$ density is below par, congregatory behaviour predominates and leads to rapid recovery; at ρ_c, minimum Δ, congregatory behaviour is maximised; at $\rho < \rho_c$ spatial recovery is greatly impeded, T_R is prolonged and relies on high reproductive rate. Density units are arbitrary as in the original data. Densities below ρ_0 are difficult to measure and short-lived, hence the data points are inadequately represented.

sophisticated behaviour, and the problem has been to find a distance function common to such diverse organisms as, for example, the migrant monarch[16] and the sedentary *Drosphila*[20], the territorial blackbird[21] and social man[13].

The working model does not deal with the underlying complexities of social behaviour[62] and bizarre adornment associated with sex[63], which can be regarded largely as visible evidence of the congregatory behaviour, nor with the equally complex behaviour and locomotory mechanisms associated with migration[25], for they are too difficult to measure. It deals with the net displacement resulting from these. When the motion is centrifugal to the nearest population concentration it is here classified as 'migratory' to distinguish it from the random motion implied by 'dispersive'. Alternatively, when the motion is centripetal fulfilling the function of "gravitational attraction" in Skellam's[64] population concept, but missing from Lidicker's[65], it is here called 'congregatory' to distinguish its active motion from the resulting instantaneous static condition or aggregation. Migratory motion, like congregation, is overlaid by elaborate behaviour patterns and can be almost obscured by movements concerned with reproduction, fighting and feeding, especially daily and seasonal commuting in man and certain highly mobile and long-lived birds whose feeding and reproductive sites are far apart[60-66]. Nor does it necessarily imply displacement right outside occupied territory, for much migration is internal. The displacement crucial to fitness is that between the birthplace of one generation and that of the next and it is the distribution of sexually mature adults as in Fig. 2 that is, therefore, relevant.

We postulate that the net centrifugal displacement of an individual (Δ_m) generated by migratory behaviour as defined above, is proportional to a fractional power of the population density (ρ) it experiences; $\Delta_m = G\rho^p$. So also is net centripetal displacement (Δ_c) due to congregatory (i.e. anti-migratory) behaviour; $\Delta_c = H\rho^q$. The exponents p and q are rate constants for density-dependent migration and congregation respectively which may be specific. G and H are constants of proportion for which we offer no biological interpretation.

If we adopt the sign convention that any tendency to increase the mean distance between individuals is positive while reduction in mean separation is negative, the intuitive sum of the two conflicting density-dependent behaviour sets is

$$\Delta = G\rho^p - H\rho^q \tag{2}$$

Equation (2) may be written in the parametric form

$$\Delta = \varepsilon\left[\left(\frac{\rho}{\rho_0}\right)^p - \left(\frac{\rho}{\rho_0}\right)^q\right] \tag{3}$$

where G and H are replaced by the biologically identifiable parameter ρ_0, the density at which migration exactly balances congregation[67], and

$$\varepsilon = (H^p G^{-q})^{1/(p-q)} \tag{4}$$

which is a scale factor containing the dimensional units. Congregation (negative displacement) now occurs when $\rho < \rho_0$, and migration when $\rho > \rho_0$ (see Fig. 4). The condition $p < q$ cannot evolve because growing populations would continue to congregate indefinitely and condense into a point in space.

Simulation and interpretation

For simplicity in the primitive simulations presented here (Fig. 5), reproduction is by binary fission at random in space among those individuals reproductively mature, and at regular intervals in time. In an environment consisting of a permanent two-dimensional random mosaic of areas that are lethal, or tolerable but prevent reproductive maturation, or hospitable, the individuals of a founder population divide and each daughter organism responds, by movement, to the population density at the time of birth. This Δ-response is to the density at a fixed population centre and is assumed to be radial. Mortality occurs only when movement ends in a hostile part of the environment. In practice the Δ-response may be to the individual's or to its parent's experience, to the distance from the nearest neighbour, or to number of social contacts, level of aggression or competition, or to other epideictic[56] or environmental cues relating to population density, and may not be radial. In a few generations population nuclei on hospitable areas reach a quasi-stable condition when migration balances natality (Fig. 5*a*). Satellite populations, repeating the process by responding to their own population centres, would ultimately occupy all the habitable areas.

Successive simulations with the environmental mosaic changing between generations (not shown here) generate additional movement and mortality. The resulting redistribution

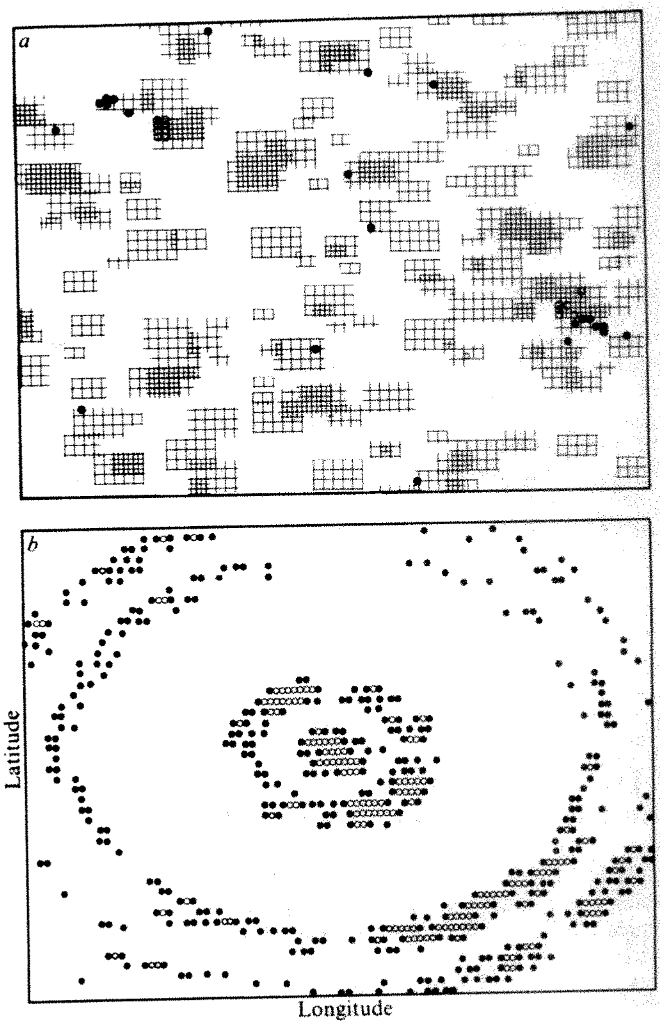

Fig. 5 *a*. Simulated population complex of a highly migrant species, something like an aphid, founded by three individuals and quasi-stable after five generations: Δ-response parameters, $\varepsilon = 0.333$; $\rho_0 = 40$; $p = 4$; $q = 2$, in an unchanging heterogeneous, mainly hostile, environment. Blank background is hostile, faint hatching tolerant but unproductive, denser hatching permits reproduction. At this stage two nuclei are reproducing and excess offspring migrate. Individuals on non-reproductive sites await an environmental change that will either release reproduction or kill them; their prospects depend on Southwood's[46] τ/H ratio, currently being held in suspense by the models fixed environment. *b*, When the environment is homogeneous and the universe unlimited, density annuli radiate outwards from a point source in successive synchronised generations; parameters similar to (*a*). In this form the model describes the annular pattern of movement familiar, on a short time scale, as the radar ring angels[76] produced by the commuting flight of starlings in response to a daily cycle in the changing balance between feeding and flocking behaviours. The figures are distorted to conserve space. Hollow circles are occupied by more than one individual.

of population centres becomes a game of hide-and-seek, emulating the response of local population elements to predation, disease and other restraints, akin to Huffaker's survival by refuge[68]. The net area population density now depends on the balance between rates of environmental change, and the movement and reproductive rates of the organism, which fails to use some resources because they are not disovered in time. Hence the population is stabilised below carrying capacity. Environmental change releases the reproductive potential of surplus individuals that wait in survival areas to mature if conditions improve, or to die if they worsen. The balance is set by the parameters in the Δ-function with respect to the environment into which further non-random elements are introduced because the Malthusian restraints are themselves biological and hence non-random[36,69]. This now generates further local contraction[70] and small-scale geographical shifts of density like those clearly visible in successive years in Fig. 2.

The simplifying conditions adopted for simulation appear not to be restrictive. Age-dependent or facultative Δ-behaviour and sexual reproduction add complexity in the computing but without fundamental change in the outcome, so far as we can tell, for the resulting distance function appears to apply quite well to sexual animals with high levels of organisation (Fig. 4). Behaviour inherited or imprinted from the parents' or grandparents' experience or modified by experience throughout life, further complicates the computing but seems not to affect the outcome. Refinements of social and migratory behaviour, together with increase in size, minimise migratory risks by providing more accurate sensory information on which to make the choice. Again parental care and altruism add sophistication but seemingly make no fundamental change in the logic. Non-radial migration, by controlled orientation or navigation, or even environmentally assisted motion, affects individual migratory risks and hence proportionate mortality. It affects the ultimate spatial disposition only in large-scale geographical trends, because final settling place depends on the successful location of environmental holes, not just on navigation[71]. Death due solely to aging is a minor factor and all other causes are included in environmental adversity.

If each new recruit responds independently to density, a continuous generation model is formulated; synchronous response generates a discrete system as in the examples shown here.

The ecological results and the evolutionary consequences

Analysis of the spatial dispositions generated by simulation yields the power relation between variance and mean population density in equation (1) that is required by the field evidence (see Fig. 3). While the power relation could arise from mathematical models based on other functional mechanisms, this result suggests that our conceptual model for a balance between specific distributive behaviours is reasonable.

The Δ-function for individuals results in a density distributing process which generates a hollow frequency distribution of numbers against displacement in a population which matches the experimental evidence on dispersal for organisms as disparate in behaviour as *Drosophila* and man (see Fig. 4). Again, other functional mechanisms could generate appropriate distributions but no previous model has comprehended spatial behaviour over so wide a range of distances or organisms. We suggest that, if migration and congregation are seen in the collective sense used here, the dispersive behaviour of other animals and plants[72,73] will be found to fit the pattern. The non-migratory condition when $\rho < \rho_o$ is perhaps most readily recognised in island life where ρ_o is high due, for example in insects, to flight-inhibiting behaviour and even morphological adaptation such as brachyptery.

The only biological premises involved are that an organism can move at some stage in its life history before reproduction and can detect directly or indirectly, by any means, including those listed by Wynne-Edwards[56] and Wilson[62], the presence of another of its own kind and hence can respond to it. Reproductive success then often lies in the avoidance of overt competition by movement and goes to the individual with the best Δ-adaptation, rather than to the more superficially successful competitor, and so provides an essential[70,74] counterbalance to excessive competitiveness.

As with other population models, migration and mortality can in some respects be equated. The emphasis is here shifted from the key factors that cause death to the place where they operate, because this reflects the real space hostility pattern of the total environment, including other organisms, and to the subsequent replacement of individuals by movement as well as by birth. Thus the return time for populations displaced from equilibrium, T_R (ref. 8), can be short-circuited by migration. A demon with perfectly developed Δ-response and ability to locate environmental holes could survive almost any catastrophe. If survival is thus seen as a spatial exercise in eluding adversity, evolution depends on learning the geometry, not just of Hamilton's selfish herd[55], but of the changing Darwinian landscape of which the herd is a part.

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Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes

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The culture lifetime of epidermal cells of newborn humans is increased from 50 to 150 generations by adding to the medium epidermal growth factor, a polypeptide mitogen. EGF seems to delay senescence of the cells by maintaining them in a state further removed from terminal differentiation. This effect is revealed by a greater ability of the cells to survive subculture and initiate new colonies, but not necessarily by an increased growth rate.

MAMMALIAN epidermis is composed principally of a single cell type, the keratinocyte, in various stages of differentiation. The basal layer, separated by a basement membrane from the fibroblasts of the underlying dermis, contains the dividing keratinocytes. These give rise to progeny, some of which no longer divide but move outward from the basal layer, terminally differentiate, and eventually are shed from the surface.

In cell culture conditions, keratinocytes depend on the support of fibroblasts in order to initiate colony formation[1,2]. When human diploid epidermal keratinocytes obtained from foreskin of newborns are inoculated together with lethally irradiated 3T3 cells, the keratinocytes grow from single cells into macroscopic colonies. Colonies can be disaggregated to single cells with trypsin and EDTA and the cells subcultured with fresh irradiated 3T3 cells. A fraction of the keratinocytes undergoes terminal differentiation even in growing colonies; these cells leave the basal layer, increase in size, and develop an envelope resistant to detergents and reducing agents[3]. At least partly because of this terminal differentiation, colony-forming efficiency on transfer is lower than that of fibroblasts. Keratinocytes derived from foreskin of newborn humans were cultivated serially through a total of about 50 cell generations but those of older donors had a reduced culture lifetime (ref. 2 and unpublished observations). This behaviour resembles that of cultivated human fibroblasts, whose restricted culture lifetime has suggested an analogy to the process of ageing[4].

Epidermal growth factor (EGF), a polypeptide with molecular weight 6045 and a known amino acid sequence[5] was first isolated from the male mouse submaxillary gland by S. Cohen, and found to enhance growth and maturation of epidermis in newborn mice[6,7], and in organ cultures of epithelial tissues[8,9,10,11]. It has more recently been found to be an extremely potent mitogen for fibroblasts[12,13,14], and for some other cell types (refs 15, 16, and R. Holley, personal communication). Most cells bind EGF with a K_m of ~ 1 ng ml^{-1}, a concentration similar to that present in mammalian plasma[17].

We describe here experiments on the effect of mouse EGF on cultivated epidermal keratinocytes derived from foreskin of newborn infants. The culture methods, including the use of irradiated 3T3 cells, have been described earlier[1,2,3]. For the first experiments, the EGF was kindly donated by Dr Cohen; later experiments were carried out using EGF purified by Dr T.-T. Sun according to Savage and Cohen[18]. The effects we describe have a bearing on the general problem of the lifetime of serially cultivated cells, and on possible relations between this phenomenon, ageing and terminal differentiation.

Effect of EGF on colony expansion

Culture dishes (50 mm) were inoculated with 5×10^3 epidermal keratinocytes and one-third the confluent density of lethally irradiated 3T3 cells (x3T3). As EGF was found

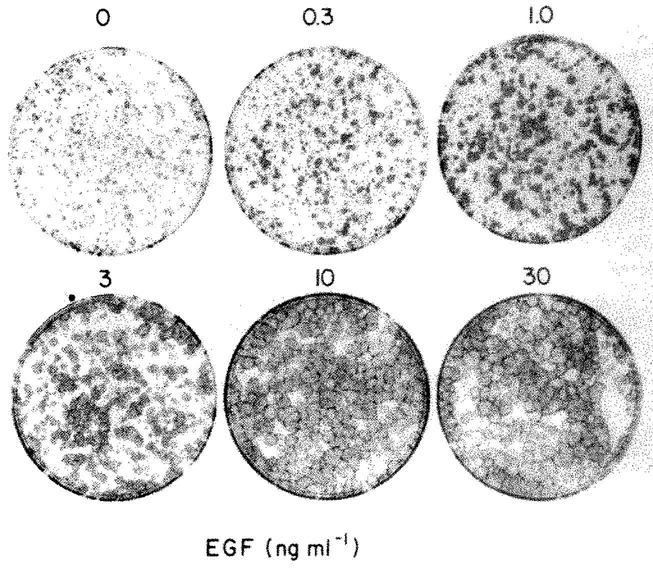


Fig. 1 Effect of EGF on the morphology and size of growing keratinocyte colonies. Dishes (60 mm) were inoculated with 5×10^3 keratinocytes of strain N at their 43rd culture generation and the Dulbecco-Vogt modification of Eagle's medium supplemented with 20% fetal calf serum and hydrocortisone, 0.4 µg ml^{-1}. EGF was added at various concentrations beginning on d 4. Cultures were fixed on d 13 and stained with Rhodanile blue[1]. Colonies grown in the presence of EGF are larger, but also thinner and the intensity of their red staining by Rhodanile blue is reduced. At higher concentrations of EGF, the colonies sometimes acquired a shredded appearance, and growth was retarded.

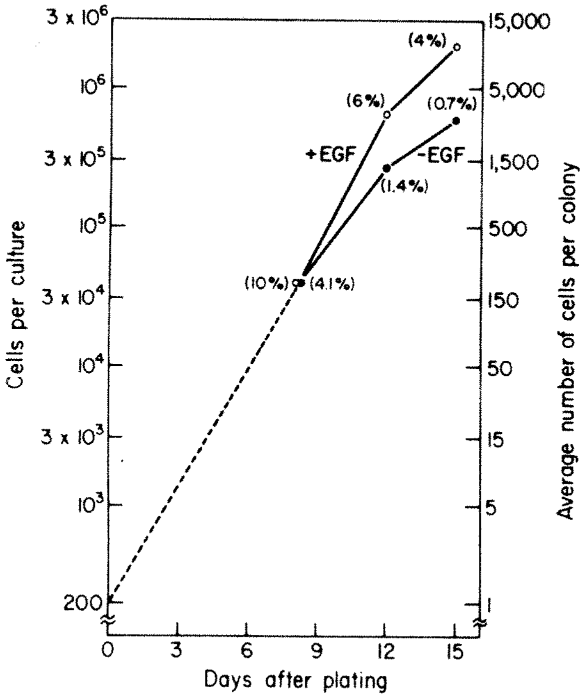


Fig. 2 Effect of EGF on growth rate and colony-forming ability of cultured human epidermal keratinocytes. Strain M. keratinocytes, at their 20th generation in culture, were plated at 1.9 × 10^4 cells (200 colony-forming units) per 60 mm dish, together with lethally irradiated 3T3 cells. Beginning at the first medium change 5 d after plating, EGF was added at 30 ng ml^{-1} to half of the cultures. Cultures were refed every 3–4 d thereafter. On d 8, d 12, and d 15 after plating, the x3T3 cells of a culture were removed with EDTA; the epidermal colonies were then dispersed to single cells with a mixture of trypsin and EDTA and counted in a haemocytometer. Aliquots were replated with x3T3 to determine colony-forming ability. ○, Cultures to which EGF was added beginning on the 5th day after inoculation; ●, no EGF. Values in parentheses give the colony-forming ability of the cells upon subculture. Cells growing in the presence of EGF have a higher colony forming ability and maintain exponential growth for a longer period.

to reduce colony-forming efficiency slightly when added at the time of inoculation, it was routinely added to cultures at the time of the first medium change, 3–5 days later. Figure 1 shows the appearance of colonies fixed and stained on the 13th day after inoculation. Colonies growing in the presence of EGF were larger, less stratified, and stained less intensely red with Rhodanile blue. These effects were evident at EGF concentrations as low as 0.3 ng ml^{-1} and increased in magnitude up to concentrations of 10 or 30 ng ml^{-1}. Part of the increase in colony size is due to enhancement of growth and part to the spreading and flattening of the cells. When EGF is added to cultures of large colonies grown in the absence of EGF, change in colony morphology is often evident as soon as 24 h later.

In contrast to the effects of EGF, fibroblast growth factor (FGF), kindly provided by Dr Denis Gospodarowicz, produced no changes in colony morphology or growth at concentrations of 1–100 ng ml^{-1}, in keeping with its activity as a growth factor only for mesenchymal cells[19].

Effect of EGF on the growth rate and colony-forming efficiency of keratinocytes

Early passage human keratinocytes were plated at low density and grown in the presence or absence of EGF at 30 ng ml^{-1}. Figure 2 shows that while the colonies were still small, they did not grow more rapidly in the presence of EGF, but as the colony size increased, exponential growth was maintained longer and by the end of the experiment (d 15) the number of cells was about four times greater in the presence of EGF. The average doubling time for the cells while in exponential growth was about 24 h, both in the presence and absence of EGF.

Some of the cells harvested for each count were replated with fresh x3T3 cells to determine their ability to start new colonies (Fig. 2, values in parentheses). The colony-forming ability of the cells fell as colonies grew larger and as their average growth rate declined, but was always greater for the cells derived from cultures grown in the presence of EGF. The difference was small at first but became progressively larger as the colonies became larger. At the end of the experiment (d 15) the difference was about sixfold.

The effect of EGF on small colonies was more closely examined by using two other strains of newborn keratinocytes. Cultures were plated with 10^5 cells of strain N or strain K, previously grown through one subculture in the absence of EGF. EGF was added to some cultures 2 d after inoculation and the growth and colony-forming ability of the cells of the two sets of cultures were compared during the following 4 d. EGF had no significant effect on growth rate (Table 1), but increased the colony-forming efficiency of the cells by 50% within 2 d of its addition and by 60% to twofold within 4 d.

These experiments suggest that the effect of EGF on the growth rate of large colonies is due to its ability to oppose inhibitory influences that begin when the cells become crowded. There are several possible ways by which this effect might be brought about, but all would have to alter either the generation time or the relative magnitude of the dividing fraction. (These alternatives are not completely independent, since an increase in the growth rate of the dividing fraction would, if the time required for terminal differentiation were unchanged, lead to an increase in the relative size of the dividing fraction.)

The fact that EGF increases colony-forming ability before the growth rate is detectably increased indicates that, even while the magnitude of the dividing fraction is unaltered, there is a change in the state of the dividing cells

Table 1 Effect of EGF on the cells of small keratinocyte colonies

Strain	Days after Inoculation	Cells per culture (x10^{-5})		Colony forming efficiency on subculture (%)	
		−EGF	+EFG	−EGF	+EGF
N (23 gens.)	2	0.74	—	9.7	—
	4	2.6	2.1	13.3	19.5
	6	11.0	9.8	7.0	11.5
K (25 gens.)	2	0.56	—	5.2	—
	4	3.4	2.7	7.6	11.3
	6	16.0	13.0	3.5	7.2

Cultures were inoculated with 10^5 keratinocytes and x3T3 cells on day zero. Cultures were refed on d 2 and EGF was added to 10 ng ml^{-1} At intervals the 3T3 cells were removed from a culture with EDTA and the keratinocytes were harvested in trypsin and EDTA. After counting the cells, some were reinoculated with x3T3 cells to determine their colony forming ability. Values are means (±15%) obtained from duplicate cultures.

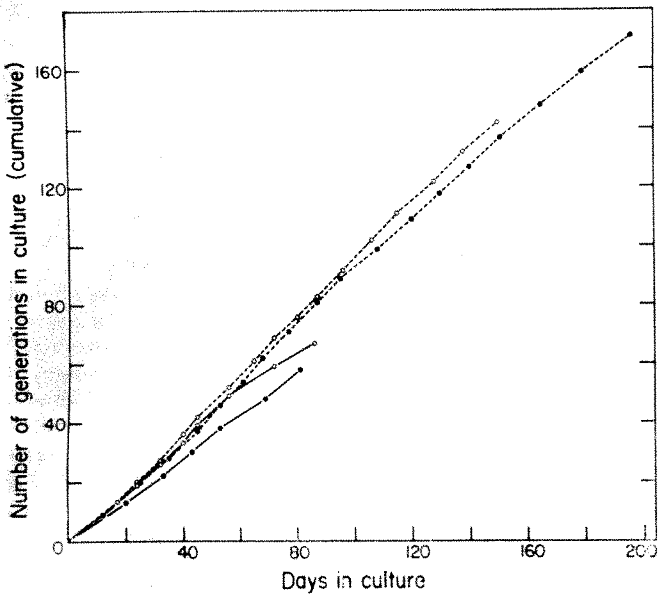


Fig. 3 Effect of EGF on the culture lifetime of human epidermal keratinocytes. The number of cell generations was calculated from the number of colonies formed and the number of cells harvested at each transfer. Each point represents a single transfer. The last point was obtained from the harvest of the last culture in which colonies formed. The average growth rate is given by the slope. – – –, Cultures grown in the presence of EGF; ——, controls grown on the absence of EGF. ●, Strain HFE; ○, strain E.

better enabling them to survive disaggregation and form new colonies. In order to have this effect, it was found that EGF must interact with cells able to proceed through the growth cycle. For example, if keratinocytes were prevented from growing by suspending them in medium stabilised with methyl cellulose, they lost their colony forming ability with a half life of 3–4 h, and later acquired markers characteristic of the terminally differentiated state. This extremely rapid loss of colony forming ability by keratinocytes, so different from the behaviour of fibroblasts, did not seem to be affected by the presence of EGF, or by growing the cells in the presence of EGF immediately before inoculating them into suspension cultures. Clearly then, keratinocytes can respond to EGF only if they are able to grow. Further data on this effect of EGF were obtained from the studies of serially cultivated cells described below.

Effect of EGF on the culture lifetime of keratinocytes

As reported earlier, the culture lifetime of newborn human epidermal keratinocytes in the absence of EGF did not exceed 50 cell generations[2]. As cultures maintained a higher proportion of colony forming cells in the presence of EGF, we examined the effect of EGF on the culture lifetime. In this experiment, contaminating dermal fibroblasts were selectively removed[3] during the first two subcultures, and therefore never overgrew the keratinocytes in later subcultures. The cultures were always transferred while the cells were in exponential growth. For these reasons the values for culture lifetime were more reproducible than those obtained in conditions used earlier[2].

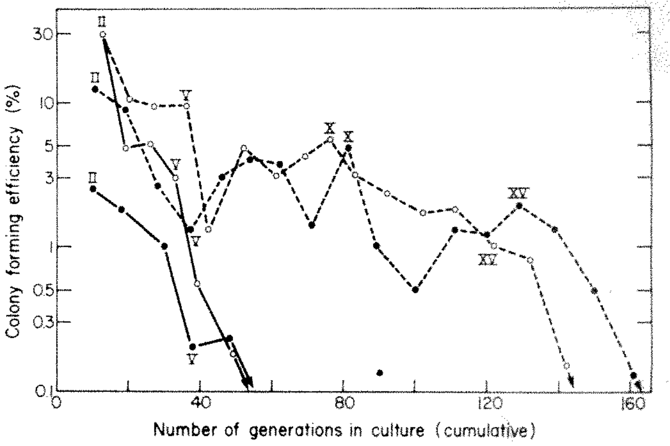
Two independent human keratinocyte strains, HFE and E, were serially transferred by inoculating 2×10^4–2×10^5 cells per 60 mm dish and subculturing 7–11 days later. EGF was added beginning with the first refeeding at 3–5 days after each inoculation. The colony forming efficiency of the cells at each subculture was determined by plating 3×10^3–3×10^4 keratinocytes and counting the number of colonies produced. All inoculations were made together with the standard density of irradiated 3T3 cells. The number of generations in each subculture was derived by relating the number of colonies (assuming each to be initiated by a single cell) to the total number of cells harvested. The number of generations so calculated is an average for the descendants of each colony-forming cell; but because of terminal differentiation in growing colonies, the dividing fraction undergoes more than the average number of doublings.

The maximum culture lifetime, as found earlier, of the two keratinocyte strains derived from newborns, and grown in the absence of EGF, was 6–7 transfers and about 50 cell generations. Addition of EGF to the medium increased the culture lifetime to 17–18 transfers and over 140 cell generations (Fig. 3) but did not significantly affect growth rate (the slope of the lines). Also, the growth rate declined very little on serial cultivation in the presence or absence of EGF until two transfers from the end of the life of the cultures. Thus the effect on culture lifetime does not depend on a change in growth rate.

When colony formation was examined at each transfer, however, large differences were apparent (Fig. 4). In the absence of EGF, the colony forming efficiency during the early transfers was in the range of 2–10%. At each transfer colony formation declined, and when it dropped below 0.1% (by about 50 generations), it was not possible to obtain growth at the next transfer. Colony formation by cells grown in the presence of EGF was higher from the very beginning, and although it declined with serial transfer, the rate of decline was much less than in the absence of EGF. The efficiency was still about 1% after 16 transfers, when both strains had reached 130 cell generations and were close to the end of their culture lifetime. An orcein stained chromosome preparation of strain HFE at 140 generations revealed a diploid chromosome number and no obvious abnormalities.

The number of keratinocytes which can be generated in culture through serial transfer depends not only on the number of cell generations the population can be grown but also on the plating efficiency at each transfer. In the absence of EGF, the possible expansion in culture of the keratinocyte population of newborns does not exceed a factor of 10^6. Since EGF increases both lifetime and plating efficiency, the corresponding value exceeds 10^{16}. Clearly, if large numbers of culture grown keratinocytes have any practical use such as for grafting, EGF would be valuable for the cultivation.

Fig. 4 The effect of EGF on colony formation during the culture lifetime of human epidermal keratinocytes. – – – –, Cultures grown in the presence of EGF; ——, cultures grown in the absence of EGF. ●, Strain HFE; ○, strain E. Roman numerals give transfer numbers. Each value for colony forming efficiency was derived by inoculating cells at 2 or 3 dilutions, so as to obtain at least two dishes containing 50–300 colonies.



Significance of the restricted lifetime of cultured mammalian cells

The existence of a reproducible lifetime of cultured human diploid fibroblasts[20] has suggested an analogy to the process of ageing and has led to many studies on the phenomenon, as well as to theories of its basis[4,21]. Although a reproducible lifetime can be obtained for fibroblasts under standard conditions, the lifetime can be increased by improving the culture conditions[22,23,24]. This would not be expected if the lifetime were limited by a fixed rate of mutational errors or errors in DNA replication. The dependence of the culture lifetime of diploid cells on the culture conditions is shown more clearly by keratinocytes, for we have succeeded in tripling the culture lifetime of this cell type by simply including EGF in the medium.

Although the culture lifetime of human keratinocytes in the presence of EGF exceeds considerably that of human fibroblasts under the best conditions, it is still far below what should be expected from the doubling time of the basal cell population *in vivo*[2]. Our method of calculating culture lifetime does not consider that some cells terminally differentiate in growing colonies and are therefore removed from the proliferating pool. Cells remaining in the pool therefore undergo more divisions than are calculated from the total cell yield per colony. Although we cannot correct for this factor, it seems likely that at least a small part of the discrepancy between culture lifetime and *in vivo* lifetime of the keratinocytes may be explained in this way.

It has been suggested[25] that restriction of the culture lifetime of fibroblasts and the depletion of colony forming smooth muscle cells of older animals are due to terminal differentiation, but no suitable marker exists to define a terminally differentiated state in these cell types. Haematopoietic cell populations, whose proliferative behaviour is usually described in terms of stem cell kinetics[26], show a decline in the number of colony forming cells during serial transplantation *in vivo*[27], a process apparently related to the increasing differentiation of the progeny.

For the cultured keratinocyte, we have ample evidence of ultimate terminal differentiation. In addition to loss of ability to form colonies, the process includes a large increase in cell size, the formation of cornified cell envelopes resistant to detergents[3], and the later development of squames whose nuclei and cytoplasmic organelles are destroyed, resulting in a final state resembling cells of the stratum corneum. Details of this differentiation will be described elsewhere; of importance here is that when growth of cultured keratinocytes is arrested, they enter this state of terminal differentiation. This is true at any stage of their culture life.

We have shown that multiplying keratinocytes can exist in different states related to their distance from obligatory terminal differentiation. That these states are revealed by the colony forming ability of the cells but not always by their growth rate is shown by two types of experiments. First, under conditions in which EGF did not increase growth rate, it increased the ability of the cells to form new colonies. Second, there was little or no decline in growth rate on serial transfer and no difference in growth rate between cultures in the presence or absence of EGF until close to the end of their culture lifetime; in contrast, colony forming ability declined to one-tenth or one-twentieth of the initial values before evident changes in growth rate, and EGF reduced this rate of loss almost from the very beginning. Subculture evidently selects against those hitherto dividing cells too close to terminal differentiation to re-enter the division cycle after transfer. We suggest that EGF extends the culture life of keratinocytes by increasing the distance of the multiplying cells from obligatory terminal differentiation.

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letters to nature

Cosmic-ray electrons and galactic radio emission—a conflict?

MANY attempts have been made in the past to deduce information of astrophysical importance from a study of the galactic non-thermal radio continuum in relation to cosmic-ray electrons observed in the neighbourhood of the Earth (refs 1 and 2 and references therein). These investigations were carried out using the cosmic ray electron data obtained from a single experiment or by making use of an average spectrum derived from the world data, although it is known that the flux values observed by different experimenters in any energy band differ by as much as a factor of 4. This in turn has led to conflicting conclusions to be drawn from the analyses of different authors. In this paper, we present a different approach for analysing the observational data based on arguments of internal consistency between each measured electron spectrum and the magnetic field strength and the dimension of the radio-emitting region required to explain the radio observations. Such an analysis makes it possible to highlight the inconsistencies associated with some of the electron measurements and permits certain inferences of cosmic ray and astrophysical interest.

We will refer first to the spectral shape of the cosmic-ray electrons expected from radio observations. It is found from the latest compilation of the radio intensity, $I(\nu)$, by A. S. Webster (personal communication) between 10 and 8,000 MHz in the direction of the anticentre, that the spectrum above 300 MHz can be well represented by a simple power law of the type $I(\upsilon) \propto \upsilon^{-\alpha}$ with $\alpha = 0.79 \pm 0.08$. On the basis of the theory of synchrotron radiation, this implies that the energy spectrum of radio-emitting electrons in interstellar space is also a simple power law of the type $j(E) = AE^{-\gamma}$, where $\gamma = 2.58 \pm 0.16$ in the energy range of roughly 4–20 GeV for a typical magnetic field strength of 4 μG perpendicular to the line of sight; here $j(E)$ expressed in particles M^{-2} sr^{-1} s^{-1} GeV^{-1}) and A is a constant. Similarly, the radio spectrum in the direction of the halo minimum has the same spectral index between 200 MHz and 8,000 MHz. From an examination of the radio spectra from these two directions, it is inferred that the simple power law for the radio-emitting electrons should extend to at least 25 GeV and perhaps, far beyond. It is known from observations on cosmic-ray electrons above 5 GeV that they are not affected by solar modulation and, therefore, we expect that the energy spectrum of electrons observed in the neighbourhood of the Earth should also have a spectral index $\gamma = 2.58 \pm 0.16$ in the energy range between about 5 GeV and at least 25 GeV.

Table 1 summarises the experimental data on cosmic ray electron spectra determined by various experimental groups[3–15]. In this table, we have listed the experiments in ascending order of their departure in standard deviation from the expected spectral index of 2.58 ± 0.16. We have also examined the spectral index in the limited energy range between 5 GeV and 30 GeV, for those experiments which cover a wider energy range, and have found it to be the same within one standard deviation of the value given in the table. It is quite encouraging to notice that most of the experimental values of γ are consistent with the radio data within about 2σ. However, the measurements of Silverberg *et al.*[14] and Meegan and Earl[15] show departures by about 3σ and 4σ respectively and are thus in conflict with the radio data.

Table 1 Observed energy spectrum of cosmic-ray electrons above 5 GeV

Reference	Energy range (GeV)	A	γ	Departure (s.d.)
Marsden *et al.*[3]	5–15	287	2.77 ± 0.42*	0.4
Anand *et al.*[4]	12–750	113	2.69 ± 0.10	0.6
Scheepmaker and Tanaka[5]	5–290	127	2.70 ± 0.10	0.6
Zatsepin[6]	7–500	95	2.70 ± 0.05	0.7
Muller and Meyer[7]	30–900	51	2.75 ± 0.10	0.9
Agrinier *et al.*[8]	8–100	24	2.30 ± 0.20	1.1
Buffington *et al.*[9]	5–63	105	2.80 ± 0.10	1.2
Freier *et al.*[10]	8–100	105	3.00 ± 0.20	1.6
Webber and Rochstroh[11]	3–20	100	3.00 ± 0.20	1.6
Matsuo *et al.*[12]	40–800	377	3.20 ± 0.30	1.8
Hovestadt *et al.*[13]	5–22	204	2.97 ± 0.13*	1.9
Silverberg *et al.*[14]	10–220	430	3.10 ± 0.08	3.2
Meegan and Earl[15]	6–64	800	3.40 ± 0.10	4.3

*In these cases A and γ have been determined by us from the data.

We next examine the implications of the absolute intensity of cosmic ray electrons measured by various groups on the basis of the observed radio flux at 1,400 MHz toward the anticentre, which is found to be about 6.7×10^{-22} W (M^{-2} sr^{-1} Hz^{-1}), after normalising the spectrum given by Webster (personal communication) at 81.5 MHz using Purton's survey[16]. This value of the frequency was chosen in order to avoid sampling of electrons below 5 GeV, for the wide range of magnetic field strength used in the analysis. We then calculated for each electron spectrum given in Table 1 the extent of the radio-emitting volume for different mean field strengths perpendicular to the line of sight needed to account for the radio flux at 1,400 MHz, by assuming that the cosmic ray electrons and the magnetic field are homogeneously distributed throughout the emitting region. We also assumed that, for those measurements in which the minimum energy given in Table 1 is much larger than 5 GeV, the same electron spectra extend down to this energy. In Fig. 1, we have plotted the calculated magnetic field strength as a function of the extent of the radio-emitting region toward the anticentre for all the electron measurements listed in Table 1. We indicate in this figure the approximate position of various spiral arms, the longitudinal component of the magnetic field (line A) in the local arm from Faraday rotation measurements[17], and a reasonable upper limit to the field strength (line B) deduced by us on the basis of the marginal detection of 2.1 ± 2.0 μG for the ambient field in the local arm using the Zeeman splitting of 21-cm hydrogen line[18].

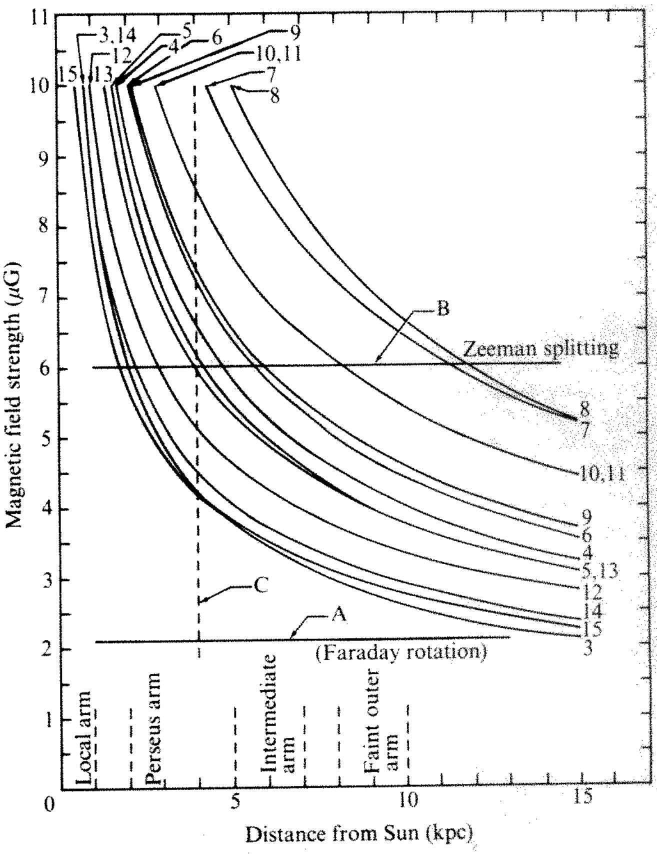

Fig. 1 The calculated mean magnetic field is plotted against the extent of the radio-emitting region toward the anticentre for various cosmic-ray electron measurements listed in Table 1 (details in text).

One observes from Fig. 1 that all the calculated curves fall above line A, thereby, demonstrating that either the magnetic field derived from Faraday rotation measurements is an underestimate or that the intensity of radio emitting electrons in the Galaxy is larger than that observed by any group listed in Table 1. However, gamma-ray observations in the energy region above 100 MeV[19] show that the interstellar intensity of nucleonic components of the cosmic rays in the few GeV region is not very different from that observed near the Earth. Therefore, we conclude from this argument that the cosmic ray electron measurements set a lower bound to the mean magnetic field of 2 μG for the interstellar space and that Faraday rotation measurements are indicative of a lower limit to the local magnetic field in the Galaxy.

It is generally recognised that the radio-emitting region toward the anticentre does not extend much beyond the Perseus arm, because the Intermediate arm itself is very faint in 21-cm observations having a gas density less than one-fifth of that of the Perseus arm[20]. We have, thus, drawn a line C in Fig. 1 at 4 kpc, as the most probable equivalent dimension of the radio-emitting region with a mean emissivity similar to that in the local arm. The intercepts of this line with the calculated curves indicate that the required field strength varies from about 4 μG to 11.5 μG, which is two to six times higher than that deduced from Faraday rotation measurements. In particular, the measurements of Freier *et al.*[10], Webber and Rochstroh[11], Müller and Meyer[7], and Agrinier *et* *al.*[8], give values ≥ 8.5 μG, which are much higher than even the upper limit set from the Zeeman splitting measurement (line B). In other words, these measurements would imply (from their intercepts with line B) an equivalent emitting region of at least 8 kpc toward the anticentre, which is difficult to accept regardless of the uncertainties associated with the positions of spiral arms in the outer parts of the Galaxy. Such large magnetic fields also require very high gas density in order to be contained in the galactic disk[21].

In conclusion, we have shown that (1) the observed spectral index of the radio continuum in the Galaxy is in conflict with some of the cosmic-ray electron measurements[14,15]; (2) the absolute intensities of cosmic-ray electrons measured by some of the experimenters[7,8,10,11] are so low that they cannot be reconciled either with the interstellar magnetic field limits or with the extent of the galactic disk toward the anticentre; and (3) it is likely that the field strength derived from Faraday rotation measurements give only a lower limit to the local magnetic field in the Galaxy.

We thank Dr A. S. Webster for communicating his latest compilation of the radio data up to 8,000 MHz and allowing us to utilise the same before publication.

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Computation of periods of acoustical oscillations of the Sun

DISCOVERY of the regular pulsations of the Sun was reported in 1975–1976 by three groups of investigators[1-3]. It has been difficult to identify these oscillations, particularly the very stable 2 h 40 min one[1,2,4]. Having had experience in calculations of the free oscillations of gaseous–liquid Jupiter and Saturn[5], we decided to compute the periods of some acoustical modes for one of the complete solar models. We chose the Abraham and Ibem[6] Model I as a model of the interior, and a model of the convective zone from Spruit[7] which had a depth of 198,00 km; an empirical model HRSA[8] was taken as a model of the solar atmosphere.

The equations of small adiabatic oscillations and boundary conditions which we used are the same as those used for giant planets[5] and similar to those used since 1959 for the free oscillations of the Earth[9]. The equations are also similar to those usually used in stellar astrophysics[10,11], but boundary conditions have a slightly different form. Our method of computation[12] is similar to that used for the giant planets. The only difference is that here we have not used an analytical start from the centre when the equations were numerically integrated. We always started with orthogonal vectors at some distance from the centre. This technique, calling for special attention when applied, proved itself to be correct for Jupiter and Saturn as well as for the Earth. Such a technique permits the calculations of acoustical modes only, separately from gravity modes, because gravity modes are formed near the centre.

Table 1 Periods $_jT_{sn}$ of free oscillations of the Sun

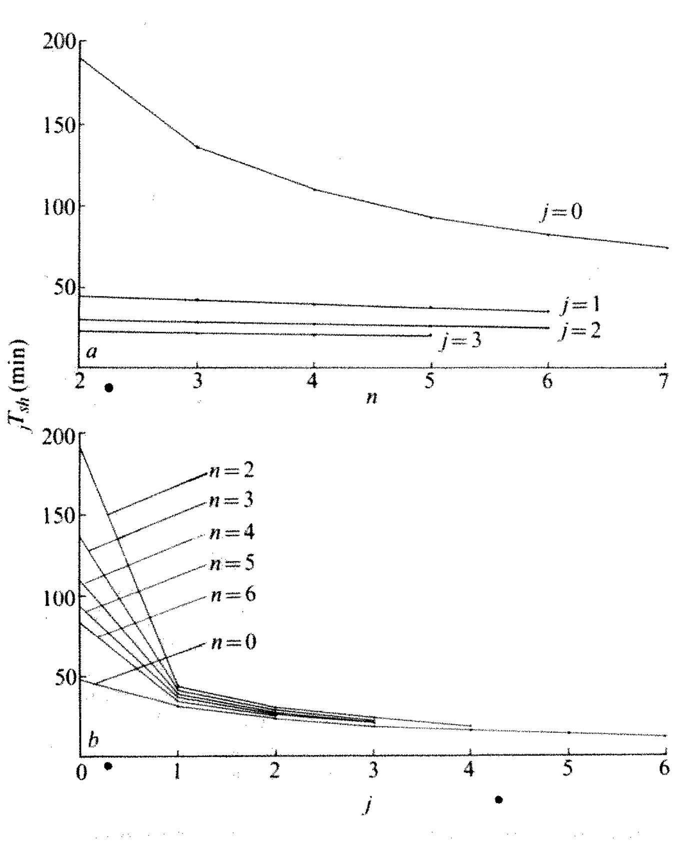
	Period (min)						
j/*n*	0	2	3	4	5	6	7
0	48	191	136	110	93.6	82.6	74.6
1	31	44	41	38.5	36.4	34.5	
2	23	30	28	27	25.5	24.5	
3	18	23	21.3	20.2	20.0		
4	—	18					
5	13						
6	11						

We report our results in Table 1 and Figs 1 and 2. The calculated periods of free oscillations are presented in Table 1. $_jT_{sn}$ denotes the period of the *j*th overtone of a spheroidal mode characterised by a spherical harmonic of degree *n*. The classification of radial modes ($n = 0$) on to overtones is that accepted for the oscillations of the Earth. The accuracy of computations is about 1 min for fundamental radial and quadrupole ($n = 2$) modes; the accuracy improves when either *n* or *j* increases.

Figure 1 shows the periods $_jT_{sn}$ as functions of both *n* and *j*.

The vector of displacements of a material point is characterised by its radial and tangential components. Figure 2 shows the tangential component *V* as a function of indimentional radius $\beta = r/R_\odot$ for the fundamental mode and the first overtones of quadrupole oscillations. The application of tangential component is more visible because of very strong attenuation of radial component with depth. For overtones V (β) has an oscillating character; index *j* determines the number of nodes.

Fig. 1 The periods $_jT_{sn}$: *a*, as functions of *n*, for the fundamental mode $j = 0$ and first overtones $j = 1$–3; *b*, as functions of *j*, for radial modes $n = 0$ and first spheroidal modes $n = 2$–6.



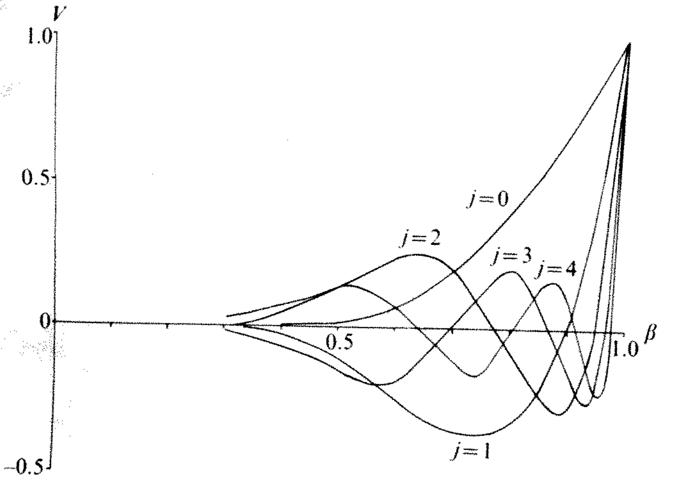


Fig. 2 The tangential component of displacements $V(\beta), \beta = r/R_\odot$, for the fundamental mode and first overtones of quadrupole oscillations.

In order to invert the procedure to improve the solar models, the first step is to identify precisely the observed oscillations. The comparison of the observed periods of oscillations of the Sun[1-3] with the computed periods in Table 1 makes it possible to assume that the mode with a period of 2 h 40 min (refs 1, 2) is the fundamental quadrupole mode, the mode with a period of 48 min (ref. 3) is the fundamental radial mode; the 40-min period[2] corresponds to the first quadrupole overtone, the 29-min period[2] corresponds either to the second quadrupole overtone or to the first radial overtone.

There are some discrepancies between our results and those of Christensen-Dalsgaard and Gough[5], and so there is another identification of the observed periods of the oscillations of the Sun. We think that the cause may be in the model. We could not examine the discrepancy in detail because we had not got the basic model for which the periods in ref. 5 were computed.

Recent calculations indicate that more accurate computations of the eigenfunctions in the inner regions may shift the periods slightly, without influencing the identification proposed.

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On presolar meteoritic sulphides

DISCOVERIES of isotopic anomalies in meteorites[1-4] inspired those same research groups to postulate nucleosynthetic inhomogeneities that were somehow carried into the early Solar System. Clayton [5,6] developed a picture treating most anomalies as extinct radioactivities trapped in mineral condensations in the expanding sites of explosive nucleosynthesis, presumably supernovae or novae[7]. As evidence for supernovae condensation grows, it becomes clear that one wants not only isotopic but also mineralogical evidence of presolar grains. What supernovae condensates bearing unique signature are likely to survive? We advance arguments here that one should search among the ubiquitous sulphides present in meteorites, searching especially for sulphides of titanium. Our reasoning is that many sulphides, especially of titanium, will not be expected in solar condensation sequences but are expected to dominate certain key zones of supernovae expansion.

The element sulphur seems to have resulted primarily from the nuclear explosions of oxygen and silicon. Woosley *et al.*[8] have analysed the arguments leading to that conclusion. The sulphur yield becomes more abundant than the oxygen fuel when about three-quarters of the oxygen has fused, and is more than 10^4 times more abundant when oxygen burning merges into silicon burning[9]. It is around this later time that the titanium abundance rises into equilibrium with those of Si, S and Ca. Therefore, if we concentrate on those supernova shells where Ti has been synthesised, we find $S/O > 10^4$. There is also no hydrogen. The elements Mg and Al are very deficient; roughly, $Mg/Si \approx 10^{-3}$ and $Al/Si \approx 10^{-3}$ while $S/Si \approx 1/2$. Shortly thereafter, the elements Cr, Fe and Ni rise to their natural abundance, without severely affecting key elemental abundances in the range $16 < A < 48$. Only Cl, K and the neutron-rich isotopes of S, Ar and Ca fall as the iron group rises. Here it is clear that following the few years required for that nuclear explosion to cool to 2000 K (refs 6, 10) the chemical condensation will proceed along unfamiliar lines. If this expanding supernova shell is not mixed by instabilities with oxygen-rich layers, the elements Si, Ca, Ti and, in sufficiently consumed Si burning, the iron group will condense in a sulphurous atmosphere. In those circumstances, on general chemical principles, sulphur is expected to play the rôle that oxygen normally plays in model solar condensation sequences[11]. It will be of enormous interest to compute analogous supernova condensation sequences, but, because many of the necessary thermodynamic data on sulphide minerals do not seem to be readily available, the plausibility of titanium sulphides is here argued for the purposes of advancing this type of argument and of calling attention to needed mineralogical research.

In conventional solar condensation sequences[11], Ti condenses completely and early as a sequence of oxides: perovskite $CaTiO_3$ at 1647 K, Ti_3O_5 at 1393 K, and rutile TiO_2 at 1139 K, all computed at a total pressure of 10^{-3} atm. Similar sequences with small temperature decreases characterise lower nebula pressures. The Ti is locked up as oxides through the falling temperatures, and free Ti and sulphides of Ti do not exist in the equilibrium calculations. In the expansion from the silicon-burning shells of supernovae, on the other hand, the abundances of key elements stand in the approximate ratio $Si:S:Ca:Ti \approx 10:5:0.7:0.03$, so that an analogous sequence of sulphides will be expected. Since a complete set of thermodynamic properties of sulphides is currently sketchy, at present it is not possible to predict exactly such a chemical condensation sequence. Hence we confine ourselves merely to pointing out that sulphides are expected to condense. Among them, titanium sulphides are especially interesting because their chemical properties, such as insolubility in H_2O, HCl, HF and solubility in concentrated HNO_3, closely resemble those of the puzzling mineral Q comprising less than 10^{-3} of the carbonaceous chondrite Allende[12]. Q is presumed to be a double sulphide of Fe and Cr, which can also easily condense in the silicon-burning ejecta. In fact, in late silicon burning, when Fe/S becomes greater than unity, most of the S may condense as FeS, so that some of the common meteoritic troilite may also have presolar origin. We concentrate instead on the titanium sulphides in the belief that they will not be synthesised in the solar condensation sequence.

Metallic sulphides grains, as postulated above, will not alone be diagnostic of condensation in a supernova-shell, as these can be formed in carbon star atmospheres too. While detailed chemical thermodynamic calculations are required to reveal the mineralogical differences between the two grains, an isotopic analysis may help distinguish between the two. For example, the most straightforward prediction from middle-to-late silicon burning is that presolar sulphides of Ti, Cr and Fe should be deficient in the two heaviest isotopes of S; that is, they should ideally be pure ^{32}S, although astrophysical instabilities may well mix the idealised layers. Early in silicon burning, however, when ^{33}S and ^{34}S are normal, a large fraction of Ti is concentrated into the light isotope ^{46}Ti; therefore, presolar titanium sulphides rich in ^{46}Ti are also to be expected when accompanied by normal S. Similarly, if presolar calcium sulphides survive, one finds that $^{34}S/^{32}S$ correlates with $^{42}Ca/^{40}Ca$, being normal early in the burning and falling to zero as the iron group rises. So our immediate suggestion is that the ubiquitous strange sulphides, especially containing Ti, are good candidates for isotopic analysis.

In suggesting that strange sulphide minerals may provide mineralogical evidence of presolar grains, we are assuming that solar condensation does not yield the same minerals. In the case of titanium, it seems likely that the refractory oxides made in the solar sequence will not be reduced later by H_2S, in which case it seems that no titanium sulphides are possible in the usual picture. Lord[13] has made this conclusion, but it may warrant a modern attack if the sulphides can be found. At present we offer this discussion in the belief that it could lead to important discoveries for the sciences of nucleosynthesis and the origin of the Solar System.

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The first metamorphic sodic amphibole identified from the Newfoundland Appalachians—its occurrence, composition and possible tectonic implications

MODELS for the emplacement of the western Newfoundland ophiolite suites include obduction over an eastward-dipping subduction zone[1], but no blueschist facies assemblages, considered to form in response to the high P/T gradients associated with subduction, have yet been identified in this area. Magnesioriebeckite and crossite which occur in metavolcanics from the vicinity of Croque, northwestern Newfoundland, provide the first indication that these conditions may have been approached. This report deals with the petrographic relations, composition and probable conditions of formation of the Newfoundland sodic amphiboles, the latest in a series of occurrences described from Vermont[2,3], Quebec[4] and New Brunswick[5,6] (Fig. 1).

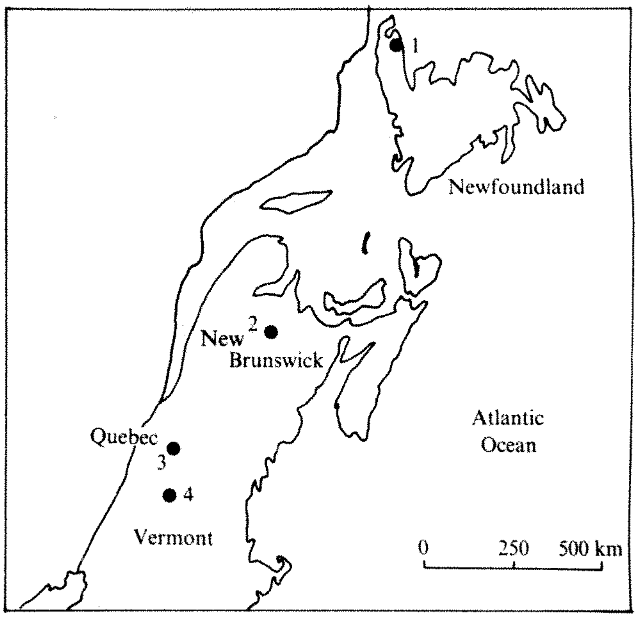

Fig. 1 A geographical location map showing the occurrence of sodic amphibole in the northern Appalachians. 1, Maiden Point Formation volcanics, Croque, Newfoundland (this paper); 2, Tetagouche Group Volcanics, New Brunswick[5,6]; 3, Tibbet Hill Volcanics, Quebec[4]; 4, Hazen's Notch Formation, Vermont[2,3].

The host rocks are undeformed metabasalts which occur at the structural base of the Maiden Point Formation, at the bottom of the Hare Bay Allochthon, a sequence of sedimentary, metamorphic and igneous rocks which includes a partial ophiolite at its top[7]. The greenschist facies mineral assemblage albite–epidote–actinolite–chlorite–sphene–stilpnomelane–magnetite–quartz–(white mica) replaces the original pyroxene and plagioclase. There is little or no preferred orientation of the minerals, and the outlines of the pre-existing plagioclase phenocrysts are often preserved. Actinolite and brown stilpnomelane appear to post-date all other phases, growing as well developed

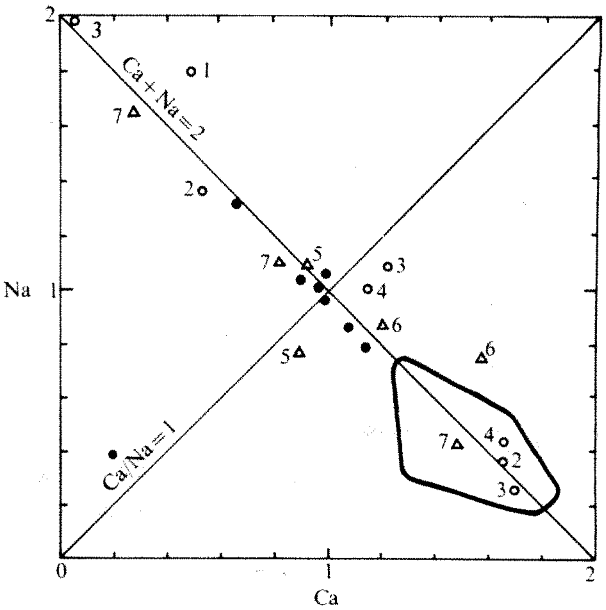

Fig. 2 The compositional range in terms of sodium and calcium content of amphiboles from the Croque metavolcanics (●). The outlined field includes all actinolite compositions from the area. Also plotted are amphibole compositions from elsewhere in the Appalachians (○) and other intermediate sodic–calcic compositions (△). 1, New Brunswick (H. Helmstaedt, private communication); 2, Quebec[4]; 3, Vermont (representative analyses of end members from the Tillotson Peak area, (J. Laird, private communication); 4, Vermont[3]; 5, Labrador[13]; 6, Switzerland[10]; 7, Washington[14].

acicular or sheaflike grains across a fine-grained mat of chlorite, epidote, albite and sphene. Actinolite and stilpnomelane do not appear in the matrix. Rare veinlets of albite, epidote and quartz transect the groundmass.

Amphibole with the pleochroic scheme X=yellow-green, Y=blue-violet, Z=indigo occurs in three samples from a suite originally collected by Smyth[7]. It rarely forms discrete grains, generally occurring as irregular zones or patches within actinolite crystals. Where a regular relationship can be defined, the blue amphibole tends to occur as cores rimmed by actinolite. No sharp boundaries between the actinolite and the sodic amphibole grains are visible. Where both amphiboles are present, actinolite is always the more abundant phase.

Table 1 Representative analyses of amphiboles from the Croque metavolcanics

	Magnesio-riebeckite RS 78–69/4-4	Intermediate sodic–calcic amphibole RS 71–172/1-3	Actinolite RS 165–70/2-3
SiO_2	52.59	51.57	54.95
TiO_2	0.06	0.05	0.02
Al_2O_3	2.57	4.85	1.16
Fe_2O_3*	7.69	6.66	2.66
FeO	15.52	11.67	11.56
MnO	0.30	0.59	0.31
MgO	9.18	12.17	14.96
CaO	4.09	7.32	9.89
Na_2O	4.57	2.82	1.63
K_2O	0.05	0.07	0.05
Total	96.62	97.77	97.19
Structural formula on the basis of 23 oxygens			
Si	7.84	7.49	7.93
Al^{iv}	0.16	0.51	0.07
Al^{vi}	0.29	0.32	0.12
Ti	0.01	0.01	0.00
Fe^{3+}*	0.86	0.73	0.29
Fe^{2+}	1.94	1.42	1.39
Mn	0.04	0.07	0.04
Mg	2.04	2.64	3.22
Ca	0.65	1.14	1.53
Na	1.32	0.79	0.46
K	0.01	0.01	0.01

*Ferric iron was estimated according to the method of Papike *et al.*[20]

Microprobe analyses show that the amphiboles range continuously from sodic to calcic compositions (Table 1). The calcium-rich amphiboles have the low Al content and nearly ideal M4 site occupancy (Ca+Na=2.0) that identify them as actinolites[8] (Fig. 2). Approximately one-quarter of the analysed actinolites are unusually rich in sodium, with $1.2 < Ca < 1.5$ formula units, and are transitional into sodic compositions, with $Ca < 1.2$ units. The sodic amphiboles are best described as magnesioriebeckite (Fig. 3), with one analysed grain containing enough octahedral Al to be classified as crossite. Although the range of compositions between actinolite and magnesioriebeckite appears to be continuous when all the analyses are considered, each of the three samples studied displays a somewhat different set of compositions. RS 78–69 contains no analysed grains with $1.0 < Ca < 1.4$, RS 71–172 has a complete range between 1.0 and 1.8 Ca formula units, and RS 165–70 contains no amphibole with $Ca < 1.2$ units.

Studies of amphiboles from blueschist facies terrains have pointed to the existence of a miscibility gap between sodic and calcic compositions[9–13] and intermediate compositions, while rare, are not unknown (Fig. 2). Klein[10] cites one case of coexisting hornblende and sodic actinolite from a metamorphic ophiolite in the Swiss Alps. Because of lack of evidence for equilibrium coexistence and the associated epidote–amphibolite facies mineralogy, this pair was thought to have formed at pressures and temperatures above the proposed miscibility gap. Metamorphic conditions of $P > 6$–10 kbar and $T < 600$ °C were estimated for the formation of intermediate 'riebeckite–tremolite' from a metamorphosed iron formation in Labrador[13]. Amphiboles from the Shuksan region, Washington, show continuous zoning from crossite to actinolite[14]. In this case, Brown concluded that with increasing dT/dP the pair sodic amphibole—calcic amphibole is replaced by a single intermediate amphibole, which in turn is replaced by Na-rich actinolite and iron oxide. If this inferred relationship is correct and applicable to the Croque metavolcanics, sample RS 78–69 would have formed under conditions of relatively low dT/dP, since it is the only sample to show any gap between sodic and calcic compositions and also contains the most sodium-rich amphiboles. Samples RS 71–172, with its intermediate compositions, and RS 165–70, with its sodic actinolites, would have formed under progressively higher T/P gradients.

The presence of sodic amphibole alone is not sufficient to define a blueschist facies assemblage[15], particularly in this case where it is an uncommon phase and has a magnesioriebeckite–crossite composition rather than one close to glaucophane. The stability of magnesioriebeckite ranges from authigenic to magmatic conditions[8], and is probably very sensitive to bulk rock composition and f_{O_2} as well as pressure and temperature. The stability field of crossite has not been experimentally defined, but probably lies between those of glaucophane and magnesioriebeckite. The metamorphic conditions at Croque cannot be deduced from the presence of this phase alone, but must be related to the mineral assemblage as a whole.

Brown[16] has suggested that the reactions chlorite+K-feldspar=stilpnomelane+actinolite and chlorite+K-feldspar + magnetite = muscovite + stilpnomelane + actinolite occur within the chlorite zone of the greenschist facies, at relatively high pressures and over a wide range of temperatures. The reaction curves, with negative slopes, lie at lower pressures on a schematic diagram than the stability fields of glaucophane and lawsonite, although they may intersect the blueschist facies curves at high pressures

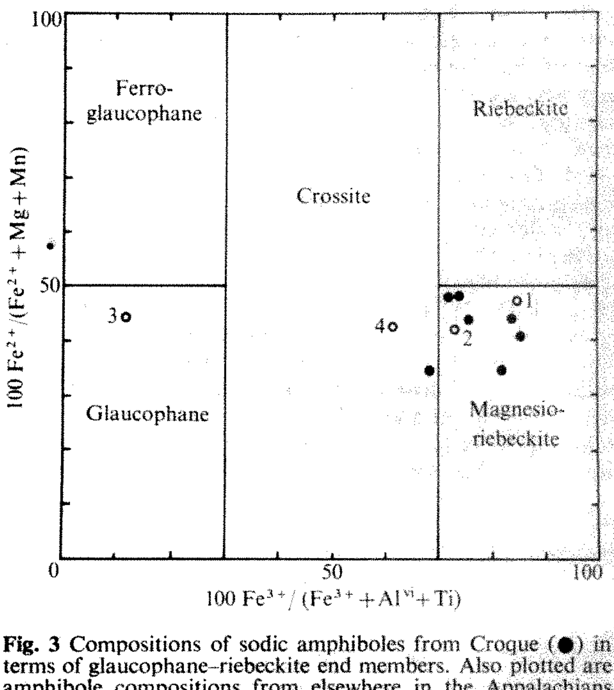

Fig. 3 Compositions of sodic amphiboles from Croque (●) in terms of glaucophane–riebeckite end members. Also plotted are amphibole compositions from elsewhere in the Appalachians (○). Ferric iron was estimated by the method of Papike *et al.*[20]. 1, New Brunswick (H. Helmstaedt, personal communication); 2, Quebec[4]; 3, Vermont (J. Laird, personal communication); 4, Vermont[3].

and very low temperatures. The mineral assemblages observed in the Croque metavolcanics seem to exemplify the proposed reaction relationships, with stilpnomelane+actinolite overgrowing the earlier chlorite-rich greenschist facies assemblage. White mica replaces feldspar in at least one example. On this basis it seems likely that the assemblages formed within the greenschist facies at rather high pressures, with one sample representing a possible transition to blueschist facies conditions.

Magnesioriebeckite occurs in comparable stratigraphic positions elsewhere in the Canadian Appalachians. Trzcienski[4] speculated, on the basis of 'crossite' in Tibbet Hill Volcanics of Quebec, that blueschist facies metamorphism had occurred in association with a subduction zone. However, Fig. 3 shows that the sodic amphibole in question is magnesioriebeckite, similar to the Croque amphiboles in composition. The presence of chlorite, stilpnomelane, actinolite and white mica in the assemblage, together with the similar stratigraphic position in volcanics which underlie a Cambro-Ordovician flysch sequence strongly suggest that the metamorphism occurred under conditions very similar to those in western Newfoundland. Magnesioriebeckite has also been observed in similar assemblages in Lower Palaeozoic volcanics in the New Brunswick Appalachians[5,6]. An amphibole with optical properties very similar to the sodic amphiboles in the Croque assemblage has been observed replacing chlorite in blocks of greywacke from the basal melange of the Humber Arm Allochthon in southwestern Newfoundland (R. K. Stevens, private communication). Glaucophane, barroisite and omphacite associated with Taconic metamorphism in Vermont[2,3] suggest that a higher P/T gradient was achieved in that region.

The structural position of the Croque metavolcanics and the lack of a tectonic fabric are consistent with metamorphism resulting from deep burial. However, only 3.4 km of overlying material can presently be accounted for in the structural sequence[17], although the mineral assemblages suggest that pressures of at least 3–4 kbar were involved. Current models for the onset of obduction in western Newfoundland would predict initial deep burial of the continental margin in response to loading by the overriding oceanic plate[18]. Later isostatic rebound and collapse of the continental margin sequence into the series of thrust sheets which is now observed would lead to the emplacement of the volcanics and associated greywackes into their present structural position. This model is supported by the presence of eclogitic dykes in the underlying, more deeply buried, Grenville basement to the east[19]. It would, however, represent conditions with lower P/T gradients than those reflected in the Franciscan 'subduction melange' of California and similar sequences where complete blueschist facies assemblages are common.

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Global climate change during the 13,500-b.p. Gothenburg geomagnetic excursion

ROBERTS and Olsen[1] suggested that high latitude atmospheric ionisation has an important role in nucleating cirrus clouds. And Harrison and Prospero[2] deduce that, if this is correct, severe drops of the Earth's magnetic field strength, such as are believed to accompany reversals, would lead to increased upper atmospheric cloudiness at all latitudes and correspondingly to major climatic events. There is likely to be warming at high latitudes but considerable cooling in the middle latitudes, in a way analogous to the aerosols[3].

I decided to test this idea in connection with the Gothenburg Excursion, the youngest well-documented geomagnetic event in the geological record. Mörner and Lanser[4] have shown this episode to have been short-lived, only 13,750–12,350 yr b.p., and Creer *et al.*[5] on the cores from Lake Erie, but with less precise dating, identified the excursion only to within 14,000–11,000 yr b.p. The global nature of this magnetic disturbance is indicated by other dates from the Gulf of Mexico, Japan, France and Australia, and often correlated with the poorly dated 'Laschamp' Event.

What happened at that time to global climates? While no cause and effect relationship can be claimed at this stage, it is useful to point out that a number of dramatic events 'happened' at the same time, for example (Fig. 1):

North American glacial record. The great Laurentian ice sheet was in full retreat by 15,000 b.p. and was about one-third melted. But suddenly, according to Dreimanis and Goldthwait[6] the glaciers ceased their retreat and reoccupied approximately 500,000 km^2 of southern Canada and the American mid-west. This readvance over a distance of 550 km (Carey, Port Stanley Till) took place about 14,000 b.p. with a last oscillation at 13,000–12,700 b.p. (Port Huron). A corresponding but smaller readvance is indicated in the north-European record[7], and data from New Zealand and Patagonia also suggest a return of the glaciers.

Eustatic evidence of glacier volumes. Mörner[8] gave the amplitude of the negative eustatic oscillation in the interval 13,000–14,000 b.p. as 9–12 m. Inasmuch as 1 mm of glacio-eustatic change represents 360 km^3 of sea water, a 10 m oscillation would involve 3.6×10^6 km^3 of water or 4×10^6 km^3 of ice. If correct, these observations suggest that within a thousand-year interval a volume of ice was newly formed that was equivalent to 20 times all of the world's present mountain glaciers put together and a third more than all the ice in Greenland today.

Central African rainfall and Nile discharge. During the last glacial stage Lake Victoria (head of the White Nile) became a carbonate-rich evaporating lake and did not overflow. Precipitation was likewise very low in Ethiopia and the Blue Nile was severely impoverished. The middle valley of the Nile became the limit of flow and gradually became silted up with a vast wedge of sediments that built up to a height of 40 m above the present level of the Nile bed, according to my own surveys[9]. Comparable desiccations of other African rivers such as the Niger and Senegal are now established[10]. Even the Congo was locally overwhelmed by

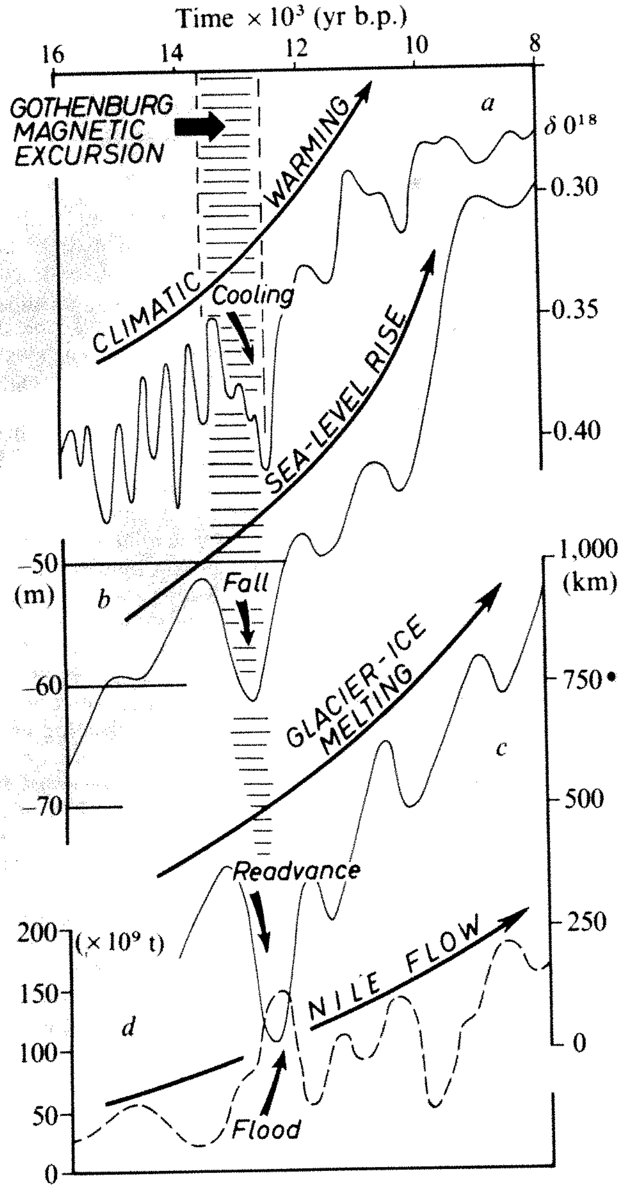

Fig. 1 Four climatic indicator curves for the period 16,000 to 8,000 b.p. The Gothenburg palaeomagnetic instability was approximately 13,750–12,350 b.p. according to Mörner and Lanser[4]. *a*, Climatic warming reflected by $^{18}O/^{16}O$ ice records from Camp Century, Greenland; *b*, sea-level rise, from data assembled by me; *c*, glacier-ice melting, measured as km of retreat in southern Canada[6]; *d*, Nile discharge, measured from the sedimentary cut and fill levels in Nubia[9].

dune sand[11]. The silt wedge of the Nile was dramatically cut through around 13,500 b.p. by an enormously augmented flow (at least four times the present discharge) and after a series of oscillations the talweg reached bedrock in many areas and the present regime was established. Sedimentation in the eastern Mediterranean seaward of the Nile delta up till around this time was a white, pelagic ooze, but then became abruptly followed by fine, terrigenous silts introduced by the Nile, which speaks for a sudden change in the fluvial regime.

It is clear from the discoveries of the last 15 yr (ref. 12) that the Milankovitch planetary insolation pattern shows a convincing correlation with gross planetary climate changes within a 92, 40, 26 × 10³ yr framework, and dynamic calculations seem to support the model[13]. Nevertheless, this theory offers us no hint of an explanation for brief climatic fluctuations or excursions on the scale of decades up to 5×10^3 yr.

The evidence of the Gothenburg fluctuation suggests that a major change in the normal global heat transfer was involved that seems to call for a non-steady state explanation. As recently recalculated by Berger[14], there was also a critical seasonality change affecting the 'sensitive' latitudes at about the same time as the Gothenburg excursion. This time was roughly about the mid-point in the gigantic melting that followed the last glacial maximum. It is difficult to relate it in any way to that warming trend. Quite the contrary, it correlates with a very brief but intense cooling. During the great melting, which lasted 10,000 yr, the sea level rose 135 m[15] and considerably changed the geography of land and water. Could a negative feedback have caused the Gothenburg cooling? In general the changes of albedo on a warming Earth combined with increasing dimensions of oceanic areas would lead to positive feedback, thus accelerating the melting. Indeed, the resultant exchange of mass loading on the Earth may very well be the cause of the magnetic instability itself[16]. The increased pluviosity of the equatorial regions at this time suggested by the Nile and other fluvial regimes might indicate a sudden drop in the continental evaporation rate.

Another curious coincidence seems to match the timing of the Gothenburg Excursion. Kopper[17] has demonstrated that in the evolution of man, each of the key subspecies introductions or cultural boundary horizons seems to correspond with an important magnetic reversal or excursion. The Gothenburg event appears to mark the end of the Neanderthals.

Most recently another report of this magnetic excursion was obtained from Blekinge, south-east Sweden, by Noel[18], but dated by uncorrected varve chronology as 12,077–12,103 ± 150 b.p. This controversial dating may be up to 300 years too young.

In summary, it should be emphasised that this proposed magnetic–climatic relationship is strictly limited to events on a scale of up to 10³ yr and is in no way a substitute for the Milankovitch modulation of ice-age climates, although in certain instances it may play a triggering role.

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Dry years in south-east England since 1698

THE recent drought and water shortage in England has re-emphasized the need for a more detailed knowledge and better understanding of the climate of the past. Without such information it is impossible to make meaningful statistical

Table 1 Years of greatest soil moisture deficit for south-east England, 1871–1970

Rank	1	2	3	4	5	6	7	8	9	10	11
From daily data	1934	1893	1921	1938	1943	1959	1896	1961	1929	1944	1965
From monthly data	1934	1944	1893	1938	1921	1901	1965	1896	1949	1929	1943

statements about the future occurrence of such extreme events. Many meteorological statements have been made about the severity and causes of the 1976 drought, mainly in the popular press, but also in the scientific literature[1]. These statements have generally concerned precipitation; but data on precipitation alone are not necessarily good indicators of drought. In this paper we distinguish between three different kinds of drought—meteorological, hydrological and agricultural drought—noting that agricultural and hydrological drought conditions are determined, not only by rainfall, but also by evaporation and by the timing of rainfall events. Using both precipitation and evaporation data for Kew we derive a time series of soil moisture deficits which we believe gives a more rational measure of agricultural drought severity than can be obtained from precipitation data alone.

Drought conditions are not determined solely by lack of rainfall. The precise evaluation of drought severity is difficult because different people perceive drought in different ways. To many people a 'drought' is a long period with little or no rainfall; indeed this is the basis of the definition introduced by Symons[2]. A more useful impression of drought severity is given by precipitation deficiency diagrams in which accumulated precipitation is compared with the long-term average for the station[3]. Accumulated departures from the average are a measure of drought severity. We may refer to a drought defined solely by deficiency of precipitation as a 'meteorological drought'. Though interesting in its own right, this is not a particularly useful definition of drought and Rodda[4] has stated unequivocally that "... a drought definition based solely on the absence of rain is hydrologically unrealistic". To quote Rodda further: "... though the causes of drought are meteorological its definition is best made in hydrological terms".

To industry and consumers of water, a drought occurs when water supplies become low. Thus a second type of drought, 'hydrological drought', is the result of a deficiency in 'excess' rainfall over some preceding period. 'Excess' precipitation is that which is neither evaporated nor used up in restoring soil moisture deficits and is therefore available to form surface run off and/or groundwater recharge. The length of a hydrological drought depends on the size of the region and the type of water resource affected[5].

To the farmer, drought conditions are those which lead to poor crop yields. An 'agricultural drought' will therefore be determined largely by conditions which lead to stunted growth or even wilting of crop plants. These are a combination of dry soil and a continuing high level of potential evapotranspiration, which the crop is unable to maintain by withdrawing water from the soil. This combination leads to a consistently high soil moisture deficit over the growing season. Thus the average soil moisture deficit over the 4-month period May to August can be used as an index of drought severity. (It is worth noting in passing the the opposite condition, of very wet soil during the growing season is equally adverse for crop yields.)

Definitions of drought have been exhaustively reviewed by Hounam *et al.*[6] who also point out the distinctions between different types of drought. Rodda *et al.*[5] in their discussion of drought indices conclude that soil moisture deficits provide the best practical drought index. Soil moisture deficits are the result of losses of soil moisture in periods when evapotranspiration exceeds rainfall. These losses depend on the type of vegetative cover; once the soil moisture is depleted below an effective root level, evapotranspiration can only proceed very slowly, at a rate much below the potential evapotranspiration rate which would occur from a fully wet soil. To account for the influence of vegetation type, and for the differences be-

Table 2 Growing-season mean soil moisture deficits for Kew, 1698–1976

	0	1	2	3	4	5	6	7	8	9
1690									29.4	100.5
1700	109.9	93.1	66.4	13.5	103.8	113.9	64.8	107.5	68.0	51.3
1710	80.9	64.6	61.9	29.4	100.0	51.6	95.3	72.0	80.8	92.9
1720	47.8	59.1	68.9	109.8	95.7	67.4	75.6	68.5	22.6	85.3
1730	78.3	114.8	76.7	86.8	37.7	60.2	95.6	89.4	76.1	64.1
1740	86.4	86.7	98.6	108.0	105.9	57.6	76.5	98.4	86.7	97.5
1750	96.6	53.9	85.0	100.4	94.9	67.1	71.8	89.2	73.2	93.9
1760	110.8	99.2	113.7	91.9	85.8	90.9	17.7	20.3	31.6	60.6
1770	45.8	79.8	90.7	60.0	77.2	94.9	93.4	11.2	100.8	69.1
1780	98.7	115.5	16.6	75.9	63.4	108.5	97.9	82.6	100.6	43.9
1790	67.9	98.7	58.3	97.9	94.0	83.7	101.7	58.1	97.4	71.3
1800	88.7	82.6	92.1	86.2	81.0	34.0	81.9	100.0	89.1	79.6
1810	80.7	67.8	34.2	84.1	59.9	93.5	6.6	56.3	94.0	63.7
1820	72.0	45.5	88.4	75.8	21.2	73.1	92.1	92.8	59.6	46.8
1830	54.1	67.7	69.7	87.0	73.8	72.2	81.4	88.3	101.8	66.3
1840	105.1	67.6	101.7	50.3	114.9	70.8	90.2	108.4	74.4	63.0
1850	95.7	80.4	91.1	47.9	78.3	83.7	63.8	99.6	80.0	97.9
1860	9.9	94.4	36.2	99.1	106.6	97.6	69.8	83.8	111.5	81.5
1870	112.7	76.8	67.0	90.5	111.7	75.4	93.3	64.8	12.8	0.0
1880	85.5	97.0	82.2	86.1	97.3	85.2	62.1	96.6	46.6	43.5
1890	36.7	65.9	89.7	118.1	61.8	104.9	113.3	95.3	105.9	94.6
1900	102.4	115.2	82.4	0.1	85.3	91.3	98.6	58.5	79.8	83.0
1910	77.1	92.8	79.2	84.3	90.4	64.9	74.9	27.3	69.0	96.8
1920	58.4	116.5	106.4	81.7	22.9	86.0	61.2	71.5	78.9	112.5
1930	58.3	37.4	66.0	97.7	123.6	82.9	81.4	65.8	117.3	83.9
1940	99.8	41.1	92.7	112.0	121.9	87.7	24.5	86.7	111.0	112.7
1950	77.6	76.2	88.4	78.2	57.4	86.4	88.8	111.9	54.9	108.6
1960	108.4	108.4	99.6	99.8	62.9	114.0	63.8	53.9	51.9	86.8
1970	106.8	38.0	105.3	110.1	116.1	93.3	126.4			

tween actual and potential evapotranspiration, Penman[7] introduced the concept of a root constant. Penman's original work indicated that potential and actual evapotranspiration rates were effectively equal for soil moisture deficits less than $C+25$ mm (where C is the root constant (unit mm) a function of vegetation type). For greater soil moisture deficits actual evapotranspiration rates are considered to drop rapidly to about 10% of the potential rate at a soil moisture deficit of around $C+37.5$ mm. Beyond this the ratio of actual to potential evapotranspiration rate is assumed to remain at about 10%. Although there are differences due to soil type, this simple model gives remarkably good results in water balance calculations over a wide range of conditions[8].

Long homogeneous series of precipitation and evapotranspiration data for Kew going back to 1698 have been constructed and discussed by Wales-Smith[9–11]; these are the longest such series available anywhere in the world. From these data one can determine, month by month, the soil moisture deficit (D) for different types of vegetative cover. D values are generally calculated by using the method of Penman[7]. The Ministry of Agriculture and the Meteorological Office publish D values for different regions at regular intervals, and the Meteorological Office has calculated D values for Kew back to 1871 using the Penman approach as discussed by Grindley[8].

We have reconstructed monthly D values back to 1698 using using the same approach and the Kew data. For most of this period only monthly data are available so it has been necessary, in order to obtain a homogeneous series, to use monthly data throughout. It is, however, preferable to use daily precipitation and evaporation data. Grindley[8] has pointed out that, because precipitation is a sporadic process, the use of monthly data alone can produce significant errors in estimating month-end D. We have found that these errors can be reduced by dividing each month into ten segments, distributing monthly precipitation and potential evapotranspiration values evenly over these segments, and then calculating soil moisture deficits accumulated over successive segments. Using this procedure we have generated monthly values of D for both short ($C = 75$ mm) and long-rooted ($C = 200$ mm) vegetation for Kew from 1698 to 1976. (75 mm has commonly been used as a root constant for short-rooted vegetation although some authors have suggested that this value is too high[5].) The results are representative of south-east England: the Kew precipitation record correlates extremely well ($r = 0.89$) with the south-eastern England precipitation series constructed by Gray[12] for the period 1840–1970.

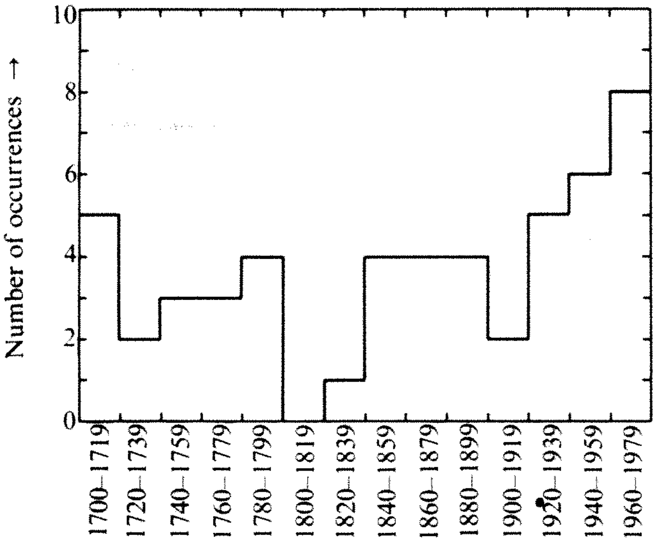

Fig. 1 Number of times the soil moisture deficit averaged over the growing season (average of month-end values for May, June, July and August) exceeded 100 mm in 20-yr intervals. The interval 1960–1979, which is still incomplete, has the highest number (8).

To test our method of soil moisture deficit calculation from monthly data we have compared our results with those calculated by the Meteorological Office using daily data. The Meteorological Office series gives the mean D over a standard catchment containing 50% short-rooted vegetation with a root constant of 75 mm, 30% long-rooted vegetation with a root constant of 200 mm, and 20% riparian land where a soil moisture deficit is never established. Using this standard catchment, the correlation between D values calculated from daily and from monthly data is extremely high; approximately 0.95 for both the 1200 month-end values and the 100 growing season (May to August) mean D values for the period 1871–1970. Furthermore, there is excellent agreement between the years with extreme D values (based on growing-season mean D) calculated using both methods. Table 1 shows the 11 'worst' years over the 100-yr test period ranked in order of decreasing mean D.

The similarity between these rankings is remarkably good when one considers that the 6th to 11th places cover a range of only 3.2 mm in mean D. The precise ranking is determined by very small differences in D. Furthermore, our results are in excellent agreement with those of Rodda[4]. His results for the period 1916–1964 show the most severe droughts as occurring in 1921, 1934, 1943 and 1944. Over the same period our data show 1921, 1934, 1938 and 1944 to be the most severe years. Rodda's results are based on data from Oxford while ours are based on data from Kew.

We have chosen to average D over the whole growing season, and to use a composite of short- and long-rooted vegetation results in order to provide a general agricultural drought index. Our index is thus a rather coarse one, and a high value will not always be an indicator of severe drought conditions for all plants. This makes a direct comparison with specific crop yields difficult, a problem which is accentuated by the influence that developments in agricultural technology have had on crop yields over the past century, which no purely environmental drought index can incorporate. Calculated growing season mean D (henceforth, D_m) values are given in Table 2.

Using this index, the 14 most severe agricultural droughts over the interval 1698–1976 are: 1705, 1731, 1762, 1781, 1844, 1893, 1901, 1921, 1934, 1938, 1944, 1965, 1974, 1976. The seven worst years are, in order, 1976, 1934, 1944, 1893, 1938, 1921 and 1974; all but one having occurred this century. 1976 shows up as the worst agricultural drought since at least 1698, although only fractionally more severe than 1934. Considering the method of constructing the series, however, it would be more realistic to say only that 1976 was among the three worst agricultural droughts during the 279-yr period of record. All three of these 'worst' droughts have occurred in the last 43 yr.

This is a strong indication that the series of D_m extremes is non-stationary. We tested this in the following manner. The series from 1707 to 1976 was divided into 27 10-yr periods and extreme D_m values for each decade were extracted and ranked. Recurrence intervals estimated by using the Gringorton[13] formula were then plotted against $(D_m)^n$ on a semi-logarithmic plot for various n. A linear fit was only possible for large n; $n = 12$ providing a good fit. From the linear fit the recurrence interval for the third highest value of D_m (1944) was estimated as 13.2 decades. In the last five decades (1927–1976) of the record, three events have occurred with equal or greater D_m (1934, 1944 and 1976). The probability of such a clustering occurring by chance, given a stationary series, is less than 0.4%. The null hypothesis that the series of decadal extremes is stationary must therefore be rejected.

An alternative estimate of the recurrence interval can be obtained by using the formula $(n+1)/m$ where n is the number of years in the record and m is the rank of a particular year[4].

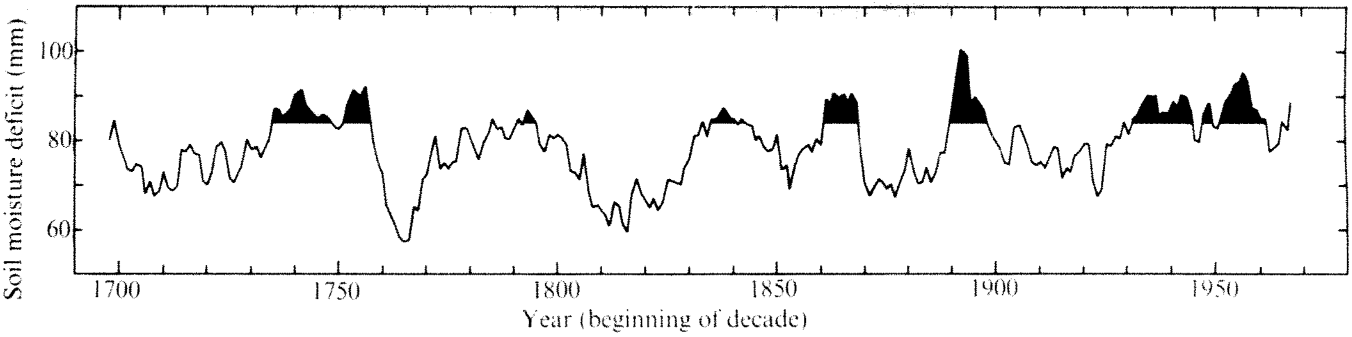

Fig. 2 Ten-yr running means of soil moisture deficit averaged over the growing season. The value of year n corresponds to the decade n to $n+9$ inclusive. An arbitrary datum level of 84 mm has been shown to accentuate the periods of higher deficits.

This gives a recurrence interval of 93.3 yr for the third highest D_m. Given a stationary series, the probability of three such events occurring in the past 50 yr is about 1.5%: a result essentially in accord with the previous analysis. The non-stationary of the extremes of the growing-season mean soil moisture deficit time series makes it extremely difficult to estimate the true return period for severe droughts.

Non-stationarity is further exemplified by the increased frequency of high D_m values this century: of the 14 worst years, 8 have occurred since 1900. This trend is illustrated in Fig. 1 which shows the number of times the growing-season mean D exceeded 100 mm in 20-yr intervals. The most noticeable features of Fig. 1 are the almost drought-free period from 1800 to 1839, and the fact that more high D_m years have occurred in the 17-yr period 1960–1976 than in any preceding 20-yr period. The drier soil conditions of this century are most probably associated with the warmer English climate which has prevailed since about 1880, although no effect of the cooling trend since 1940 is evident. This may indicate that urbanisation has had an effect on evaporation at Kew. Although we do not think such an effect can be large, it is a factor which warrants further investigation.

Another indication of the trend towards more frequent agricultural drought over the past 50 yr is given by Fig. 2 which shows 10-yr running means of average growing season D values. Figures 2 and 1 are not equivalent since the latter only isolates the extreme events. There are, however, some interesting comparisons. The recent trend to more frequent large D_m values does not show up in Fig. 2, although the period from 1930 to the present is the longest period of consistently high growing season soil moisture deficits since 1698, marginally 'worse' than that of the mid-eighteenth century.

We noted earlier that rainfall data alone do not provide a good indicator of agricultural drought. In support of this contention we found that D_m does not correlate particularly well with annual precipitation. The correlation with summer rainfall ($r = 0.80$) is somewhat better. However, in spite of this high correlation, the years of highest D_m do not in general correspond with the driest summers. This is because in addition to having low summer precipitation it is necessary to have established a high soil moisture deficit prior to the growing season, and to maintain high evapotranspiration rates during the growing season. Nevertheless, a good prediction of summer rainfall is a prerequisite for the prediction of agricultural drought: reliable rainfall estimates two to three months in advance appear to be essential.

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Structural relationships between corn yield and weather

REPORTS of crop–weather relationships are sparse in the literature and Fisher's classic paper in 1924 has not been improved upon[1]. We find this surprising in view of current concern regarding the ability of the world to feed its rapidly increasing population. This paper reports on progress in our research dealing with corn–weather relationships, a subject of general interest as corn is grown over large areas of the globe.

The data are taken from selected parts of the main corn growing region in South Africa (Fig. 1), namely, four spatially representative sub-regions in the Orange Free State. The data, for the period 1949/50 to 1964/5 consist of corn yields in 200 lb bags per morgen, monthly rainfall totals and mean temperatures at 0800 and 1400; the rainfall and temperature data apply to months over the growing season. By using the method of principal components, these data are shown to be uncorrelated[2]. Multiple regression analysis is applied to the data[3]. Yield is regressed in turn on rainfall, and temperature. The degrees of freedom dwindle, but this does not detract from the value of the strategy adopted. Time was also included in the regressions to account for any temporally varying yield stimuli not otherwise considered, such as fertiliser application. The importance of each month's rainfall and temperature in accounting for the total sum of squares in yield is shown in Figs 2 and 3. Temperature data are presented for the recording time that contributed most strongly to the sum of squares for yield; the order of inclusion of a month into the regression is indicated by the number at the top of each rectangle.

All four districts are in the summer rainfall region of the country. The yields for Bethlehem and Kroonstad show least dependence on individual monthly rainfall totals (Fig. 2). This is probably because the mean annual rainfall for these two is somewhat greater than for Bloemfontein and Hoopstad, therefore, rainfall is less inhibiting to yield at Bethlehem and Kroonstad than at the former two locations. Bloemfontein is situated furthest south and is the only region where yield is sensitive to mid-season rainfall. The yield for Bethlehem is related to both early and late rains. In the case of Hoopstad, and to a lesser extent Kroonstad, it is the late rains which are critical to yield variation.

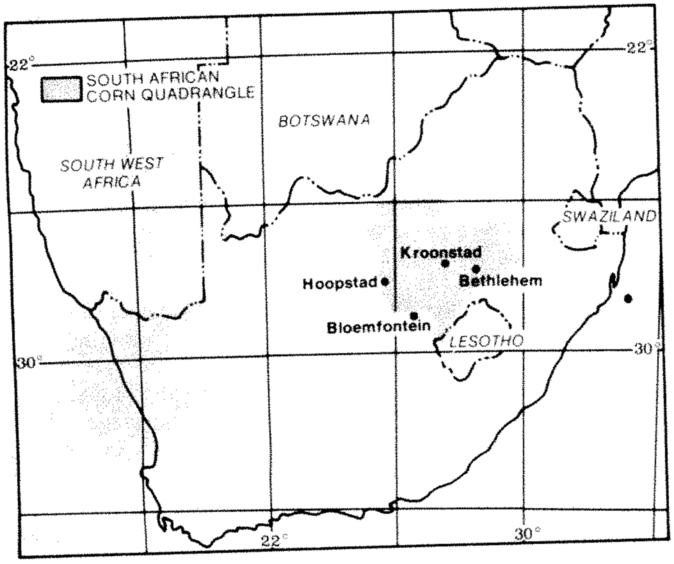
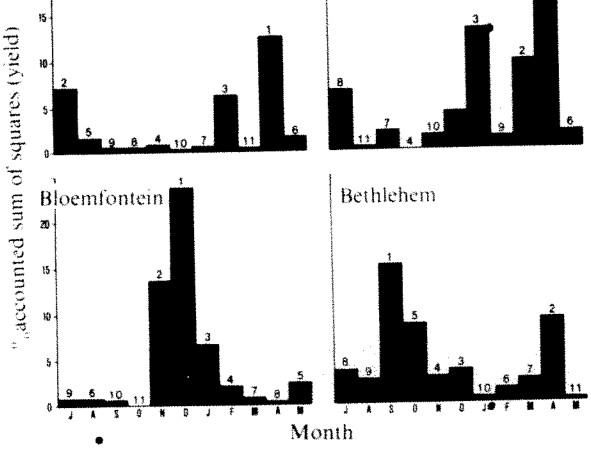


Fig. 1 The position of the South African main corn growing region (shaded), and the location of the sites considered in this paper.

The partial correlation coefficients between yield and early rains are, significantly, negative. This applies to September for Bethlehem, as well as the early months for Kroonstad and Hoopstad. The late rains, and mid-season for Bloemfontein, have positive partial correlations with yield. The adverse effects of early rains result from intense rainfall coupled with instantaneous run-off wastage.

Fig. 2 The percentage of total sum of squares for corn yield accounted for by rain over the growing season.



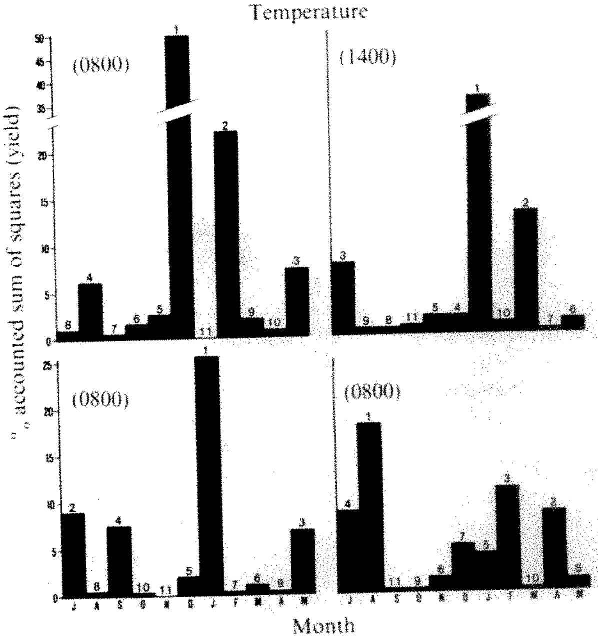


Fig. 3 The percentage of total sum of squares for corn yield accounted for by temperature over the growing season.

Yields at Bethlehem are least sensitive to temperature and when considered also in relation to rainfall, this is the most equitable region for corn. The yield has positive partial correlation with August's temperature. The mid-season temperature is important to yields in the other three regions. This applies particularly to Kroonstad which was little affected by rainfall. For this region the temperatures during December and February have negative partial correlations with corn yield while the converse is true for temperatures at the latter end of a season. This conformation is not unexpected; warm temperatures are required to assist germination, but warm temperatures concluding a season would result in loss of moisture from the crop, with consequent decreases in yield.

The most influential rainfall and temperature months were combined, and the regressions repeated. The structural relationships between yield, rainfall and temperature resulted in the following variables being drawn into the regressions. (1) Kroonstad: time, January, July, May, September and December temperatures. (2) Hoopstad: January temperature, time, March and July temperatures. (3) Bloemfontein: time, January, July, May, September and December temperatures. (4) Bethlehem: time, August temperature and September rainfall.

The percentage variance in yield accounted for by each regression is 85, 86, 95 and 70%, respectively, for factors 1, 2, 3 and 4 above. Note that, apart from time, temperature is the dominant limiting factor for yield. This emphasises the erroneous conclusions that may be drawn from an inspection of the simple correlation matrix. The advantages in using stepwise as an alternative to straightforward multiple regression become obvious. Arithmetic details relating to the analyses may be obtained from the authors.

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Methylated arsenic from marine fauna

It has long been known that certain species of marine animals contain arsenic in their tissues at concentrations up to 100 p.p.m. (ref. 1), and there is little doubt that this arsenic is naturally acquired and does not reflect environmental pollution. The toxicity of arsenic is dependent on its chemical environment and valency state[2,3]. Since the demonstration of the rapid and complete excretion by the human kidney of arsenic consumed in fish and shellfish[1,4], it has been accepted that such arsenic is in an organic and nontoxic form[5]. Clearly full toxicological evaluation of the arsenic in marine animals awaits the elucidation of the precise chemical nature of that arsenic. We have found that mussels (*Mytilus edulis planulatus* Lamarck), western rock lobster (*Panulirus longipes cygnus* George), and stingray (*Dasyatis thetidis Waite*) contain dimethylated and trimethylated arsenic together with a small proportion of inorganic arsenic.

Lobsters were collected off the western shore of Garden Island and mussels and stingray were taken from Cockburn Sound, both in Western Australia. Total soft tissue from the mussels (containing 1 p.p.m. arsenic), tail muscle from the lobster (20 p.p.m. arsenic) and muscle from the disk of the stingray (20 p.p.m. arsenic) were used. Concentrations of arsenic were determined by vapour generation atomic absorption spectrophotometry[6] after digestion of the samples in a mixture of nitric and perchloric acids. Digestion of methanol extracts of the above tissues in aqueous sodium hydroxide led, after neutralisation, to a solution from which arsines were generated on addition of sodium borohydride. Fractional distillation of these arsines from a cold trap into an atomic absorption spectrophotometer[7] led to their identification and has provided information on the alkylation pattern of arsenic contained in fish and shellfish. Sodium borohydride treatment of the methanol extracts before sodium hydroxide digestion produced no volatile arsines.

Preliminary methanol extraction was carried out on macerated tissue of each species. Virtually all arsenic was removed from rock lobster and stingray tissues in this way; 60% of that in the mussel was recovered. The extracts were each treated as follows. Methanol was removed and the residue was digested in sodium hydroxide solution. A small portion of the digest was treated, after neutralisation and addition of buffer, *p*H 4–5, with aqueous sodium borohydride. The arsines generated were trapped in a tube packed with glass beads at the temperature of liquid nitrogen. The trap was linked to an atomic absorption spectrophotometer and allowed to warm, with periodic injection of the volatilised contents of the trap into the flame, as described before[7]. Comparison of the time taken for the arsines from lobster muscle digest to volatilise and enter the flame with those for the standards—arsine, methylarsine and dimethylarsine—revealed 5% unsubstituted arsine, 60% dimethylarsine and 35% of an arsine subsequently identified as trimethylarsine. The percentages represent the constituents of the neutralised base digest when the concentration of sodium hydroxide varied from 10 to 40%: the stronger the base, the greater the release of total arsenic susceptible to reduction. Sodium hydroxide in excess of 40% did not increase the total arsenic released. Digestion was always for 15 min.

Trimethylarsine was identified by use of rock lobster tissue only. The arsines produced by borohydride reduction of the sodium hydroxide digest were trapped by passing all effluent gases through toluene. After concentration, the toluene solution was subjected to gas chromatography and the peak corresponding to the unidentified arsine was located by smell at a sniffing port (strong garlic-like odour). Trimethylarsine was identified by gas chromatography–mass spectrometry[8].

In the sodium hydroxide digest inorganic arsenic is probably present as sodium arsenate or arsenite and dimethylated arsenic as sodium dimethylarsinate. The form of the trimethylated arsenic is more doubtful. Trimethylarsine oxide seems the most likely. The possibility that trimethylated arsenic was demethylating in the alkaline digestion conditions was discounted because the proportions of di- and trimethylated arsenic did not change within the range of sodium hydroxide concentrations that brought about their release. Also, the resistance of dimethyl (trifluoromethyl) arsine to hydrolysis[9] indicated that demethylation would not occur in even the strongest basic conditions used. The close similarities in proportions of the various arsenic compounds from the different species examined may indicate the partial breakdown, in the alkaline conditions used, of a single complex arsenical. That such dissimilar species should all contain arsenic in a single form may seem more likely than that all three should contain the same proportions of three arsenicals. There are, however, no observations on which to base such speculation. Work is in progress to elucidate the chemical structures associated with the methylated arsenicals in both the methanol extract and the sodium hydroxide digest. Preliminary work on the urine of subjects consuming fish containing arsenic suggests that the arsenic remains in a bound state in the body and is excreted in that form. Work is being continued on this aspect of the study.

Although we are still some way from the stage where detailed toxicological assessment of arsenic in marine fauna can be made, it is evident that most arsenic in such situations is organically bound and the general acceptance of the nontoxic nature of this arsenic on such grounds seems to be justified.

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Jurassic labyrinthodont

The discovery of a member of the Labyrinthodontia in the Surat Basin of Queensland, Australia, brings the last known occurrence of this amphibian subclass firmly into the Jurassic.

The specimen was discovered on a property, Kolane, approximately 35 miles ENE of Taroom, within the oolitic member of the upper Evergreen Formation. As marine fossils are largely absent from Jurassic sediments of eastern Australia, the latter have been dated almost entirely by correlation of their microfloras with those of the Jurassic sequence in Western Australia the age of which has been determined by means of its marine faunas. Thus, the first appearance of *Tsugaepollenites segmentatus* and *T. dampieri* in the upper Evergreen clearly dates this formation as Upper Liassic[1,2]. In addition the possibility of an Upper Triassic age for the lower Evergreen Formation and the underlying precipice Sandstone is ruled out by the presence of *Classopollis classoides* which is not known from accurately dated pre-Jurassic sediments[1].

As far as can be ascertained before preparation, very little of the skeleton of the Kolane amphibian is missing so that, when prepared, this late survivor of the labyrinthodonts should also be one of the best known.

Many factors suggest that the specimen is a labyrinthodont amphibian. The teeth are labyrinthine in cross-section, the skull and dermal pectoral girdle are ornamented, and there are indications of sensory canal grooves on the skull. In addition, the shape of such limb or girdle bones which can be seen in cross-section is characteristic of Triassic members of the order Temnospondyli, as is the structure of the vertebrae with the neural arch detached from an enlarged intercentrum and a small unossified pleurocentrum.

The specimen is preserved in massive ironstone blocks, and preparation will present difficulties. But enough of the skull is exposed to allow determination down to familial level. The Kolane amphibian conforms to some of the most important criteria used by Welles and Estes[3] and Cosgriff[4] for inclusion of a skull in the family Brachyopidae. Its affinities within that group must remain uncertain pending its further preparation. The skull table is short and broad and on the right of the specimen the characteristic vertical plate of the pterygoid is exposed, while median to this the occipital condyle can be seen projecting well behind the level of the dorsal skull roof (Fig. 1). Orbits are well forward of the midline of the skull but are smaller than is usual in the brachyopids. The animal is big with its skull measuring more than twice the length and almost twice the breadth of the next biggest brachyopid, *Hadrokkosaurus bradyi* from the early Middle Trias[3]. A further link with that genus (which was also its closest relative in time) is that its vertebrae are of the rhachitomous type described by Welles and Estes[3] in *Hadrokkosaurus*.

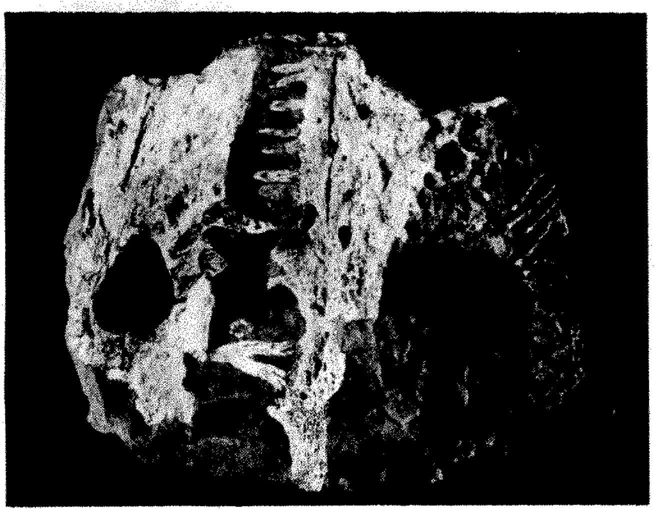

Fig. 1 Anterolateral view of part of the skull and lower jaw of the Jurassic labyrinthodont (Queensland Museum No. F7882). Part of the right orbit can be seen in the upper right hand section of the photograph. The scale measures 15 cm.

There has been one previous description of a Jurassic labyrinthodont. Longman in 1941[7] described *Austropelor wadleyi*, a jaw fragment from the Marburg Sandstone, also of Queensland, Australia. While the age of this sandstone is probably equivalent to that of the Evergreen Formation, the fact that labyrinthodonts elsewhere in the world apparently became extinct at the end of the Triassic led de Jersey[5] to postulate that this fragment must have been reworked from older sediments. Hence Colbert[6] in concluding his new interpretation of this supposed Jurassic labrinthodont states "there will have to be more definite evidence than is afforded by the single specimen of *Austropelor wadleyi* to justify the extension of the labyrinthodont amphibians into Jurassic sediments". The discovery of the Kolane amphibian has provided the evidence that Colbert sought.

Not only does this specimen extend the range of the Labyrinthodontia to the upper Lower Jurassic but it also extends the occurrence of the Brachyopidae through the Middle and Upper Triassic, and the Lower Jurassic.

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Influence of temperature on cyanogenic polymorphisms

CYANOGENIC glucosides occur widely in the plant kingdom, and in species which are polymorphic for their presence or absence they probably have ecological significance. The cyanogenic morphs may be relatively unpalatable to grazing animals[1-3] and consequently have an advantage where grazing pressure is significant[4]. Cyanogenic morphs of *Trifolium repens* and *Lotus corniculatus* are relatively rare at high altitudes, and in Europe their distribution correlates with the January 5 °C isotherm[5]. It has been inferred that where low temperatures and frost are frequent, cyanogenesis confers a reduced physiological fitness, but this aspect of the polymorphism has received less attention and has not been established experimentally. A marked difference between the morphs in their response to artificial cold treatment is shown by the data reported here, together with evidence that this difference is significant in natural conditions.

Samples from the scarp slope of the Ochil fault line in the Forth Valley, Scotland, show the effect of altitude on the frequency of cyanogenic forms of *T. repens* and *L. corniculatus* (Table 1). At each site 100–150 plants were sampled, each a minimum of 1 m apart, and tested for the simultaneous presence of cyanogenic glucosides and β-glucosidase by the picrate paper test[6]. In both species the frequency of cyanogenics decreases as altitude increases; *L. corniculatus* is absent from the valley floor in this area. That it is selection which produces this distribution can be seen by comparing the seed and adult populations. *L. corniculatus* seed was collected at each site from 100 individuals, mixed and sown in garden plots. From each plot 250 individuals were selected randomly after 6 weeks growth and tested as before. Table 2 shows that in the highland site selection operates strongly against the cyanogenic morph and in the lowland site, to a lesser extent, against the acyanogenic morph. Selection probably operates through temperature, and so its effect was tested by placing individuals of both types at +5° C and then lowering the temperature, over 2 h, to −3 °C. After 1 h at −3 °C plants

Table 1 Frequency of cyanogenic individuals at differing altitudes

Transect	Altitude (m)	*T. repens* Sample	*T. repens* % Cyanogenics	*L. corniculatus* Sample	*L. corniculatus* % Cyanogenics	Transect length (m)
Sheriffmuir	312	100	2	107	47	4,000
	168	100	20	100	94	
	30	100	62	—	—	
			$\chi^2 =$ 94*		$\chi^2 =$ 54*	
Alva Glen	305	100	5	130	67	1,200
	106	100	68	160	100	
	15	100	83	—	—	
			$\chi^2 =$ 137*		$\chi^2 =$ 62*	

*$P < 0.001$.

Table 2 Comparison of seed and adult populations of *L. corniculatus*

Altitude (m)	Seed Sample	Seed % Cyanogenics	Adult Sample	Adult % Cyanogenics	
312	250	86	107	47	$\chi^2 = 60$*
168	250	79	100	94	$\chi^2 = 11$*

*$P < 0.001$.

Table 3 Oxygen consumption as percentage of pretreatment level

Time from end of treatment (h)	Treated Cyanogenic	Treated Acyanogenic	Untreated Cyanogenic	Untreated Acyanogenic
12	199±21	139±32	105±12	101±18
24	61±31	93±61	95±18	90±16
36	30±14	79±26	87±18	105±12
48	15±15	102±13	96±12	93±10

were removed to 20 °C for 30 min and tissue was sampled at 12-h intervals. The rate of respiration of excised tissue was measured in Warburg manometers, in the dark, at 25 °C. Oxygen consumption, measured as μl of oxygen per h per g dry weight for each 12-h period was expressed as a percentage of the oxygen consumption of the same plant in the 12 h preceding treatment. Control plants received identical treatment but were maintained at 20 °C. Table 3 gives the mean values, with standard errors, of three experiments in each of which different plants were used—12 individuals altogether. Damage resulting from the cold treatment caused a marked increase in the oxygen consumption of both types, but after 48 h acyanogenics were respiring normally whereas the respiration of cyanogenics decreased below the control levels within 24 h and continued to decrease thereafter. In all individuals the treatment caused the plants to wilt, but death of all above-ground shoots occurred only in some cyanogenics; acyanogenics recovered.

Selection therefore is shown to operate against cyanogenic forms at increasing altitudes, and temperature is probably the specific factor. The interplay of temperature effects with differential grazing probably maintains the polymorphism and governs the ecology of the morphs in some areas.

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Importance–value curves and diversity indices applied to a species-rich heathland in Western Australia

RANKING of species in a community from most to least important (abundant) to yield an 'importance-value' (*I–V*) curve is a device often used by ecologists to elucidate features of that community. Increasingly in the literature[1-5], an *I–V* curve which obeys the lognormal function is being accepted as describing the ultimate in plant species diversity. South-western Australia is renowned for its apparent floristic richness and high degree of endemism. An ecological study of sand heath, considered by botanists the major repository of this supposed 'incredible richness'[6,7], has revealed a type of *I–V* curve not previously noted for vegetation. The values for six of seven indices of species diversity examined were as high as, if not higher than, those for the most diverse communities previously recorded, including the richest of tropical rainforests[2,4,8-10]. All earlier indices of equitability (evenness of species' importance) are based on models of limited applicability and a new index, based on the actual slope of the *I–V* curve, is proposed here.

The study site, situated 15.5 km south of Eneabba (29°50′S, 115°15′E), was studied in mid-spring 1975. The vegetation was low open heath[11], consisting of nanophanerophytes (68%), hemicryptophytes (20.3%), chamaephytes (5.8%) and geophytes (5.8%)[12] and had been burnt about 7 yr before. According to Raunkiaer's leaf classes, 52.6% of the species were leptophylls, 26.3% nanophylls, 18.2% microphylls and 1% were mesophylls. The average growing season for agricultural plants in the area is 4 months. The soil was highly impoverished sand over yellow clay–loam at 50–60-cm depth in a shallow valley. A representative 1,500-m transect was run across the valley and 10 equi-spaced sectors, each consisting of an average of 50 contiguous 1-m² quadrats, were examined. The 13,066 individuals encountered were assigned to 172 species and their number and canopy cover class[13] in each quadrat were noted. The total of 500 quadrats contained more than 96% of the species present

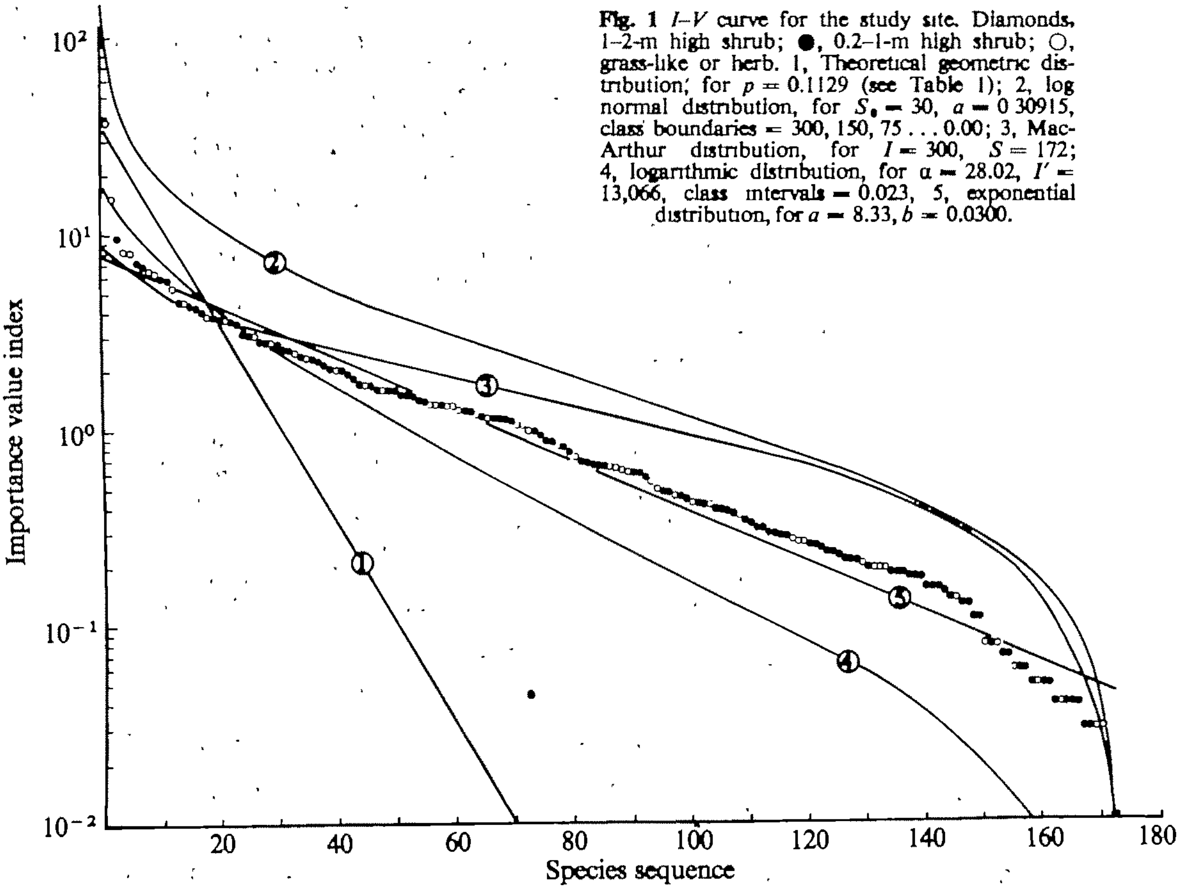

Fig. 1 *I–V* curve for the study site. Diamonds, 1–2-m high shrub; ●, 0.2–1-m high shrub; ○, grass-like or herb. 1, Theoretical geometric distribution; for $p = 0.1129$ (see Table 1); 2, log normal distribution, for $S_0 = 30$, $a = 0.30915$, class boundaries = 300, 150, 75 . . . 0.00; 3, MacArthur distribution, for $I = 300$, $S = 172$; 4, logarithmic distribution, for $\alpha = 28.02$, $I' = 13{,}066$, class intervals = 0.023, 5, exponential distribution, for $a = 8.33$, $b = 0.0300$.

in a 150-ha area about the transect, though this low heath extends, with some taxonomic changes, for hundreds of km².

Species' importance was based on the importance value index (IVI) of Curtish and McIntosh[14-16], as follows:

$$I_r = 0.1\sum_1^{10}\left(\frac{100f_r}{\Sigma_1^S f_i}+\frac{100d_r}{\Sigma_1^S d_i}+\frac{100c_r}{\Sigma_1^S c_i}\right)$$

where I_r is the mean IVI of the rth species for the ten sectors, f is the frequency of occurrence in the 50 quadrats, d is the number of individuals per sector, c is the total canopy cover per sector and S is the number of species in the sector. From this formula, the total importance value $(I) = \Sigma I_i = 300$. Though mathematically questionable, this measure was chosen to avoid bias in the exclusive use of any one index of abundance.

In Fig. 1, four mathematical distribution models often used to interpret empirical *I–V* curves have been superimposed on the data obtained for the study site. As expected from its low level of species' dominance, the vegetation had little in common with the geometric distribution[17] ($P<0.001$, Table 1). Though the lognormal curve[18] had a similar shape to the *I–V* curve, the importance of any species was always considerably less than predicted by this distribution ($P<0.001$) due to the leftward displacement of the modal interval. The presence of the complete untruncated curve using the lognormal model, however, confirms that the samples provided a good estimate of the number of species in the community[19]. The MacArthur

Table 1 Goodness of fit of various mathematical models to the *I-V* curve for the study site

Distribution	Formula	χ^2 value*	P	D_{max}†	P
Geometric	$\hat{I}_r = I_1(1-p_1)^{r-1}$	∞	<0.001	0.4134	<0.001
Lognormal	$\Delta\hat{S}_R = S_0/\exp\left(\frac{S_0\sqrt{(\pi R)}}{S}\right)^2$	422.78	<0.001	2.0253	<0.001
MacArthur	$\hat{I}_r = \frac{I}{S}\sum_r^S\frac{1}{i}$	116.72	>0.05	0.1639 (I_1 omitted: 0.0853	<0.001 >0.05)
Exponential (S_1-S_s)	$\hat{I}_r = a\exp(-bS_r)$	97.78	>0.05	0.1442	<0.001
Logarithmic	$\Delta\hat{S}'_R = \alpha\left(\frac{I'}{I'+\alpha}\right)^{R-1}/R$	73.16	>0.05	0.0622	>0.05
Exponential ($S_{10\%+1}-S_{90\%-1}$)	$\hat{I}_r = a\exp(-bS_r)$	0.57	>0.05	0.0100	>0.05

$\hat{I}_r$ = estimated IVI for the rth species, $p_1 = I_1/\Sigma I_i$, $\Delta\hat{S}_R$ = estimated number of species in an IVI interval R intervals from the modal interval with S_0 species, S = total number of species in the collection, a = constant (I_0), b = constant (slope), $S_r = r$, $\Delta\hat{S}'_R$ = estimated number of species in an IVI interval corresponding to R individuals, $\alpha = S/\log_e(1+I'/\alpha)$ where I' = total number of individuals.

*χ^2 test on 170 d.f., $\chi^2_{0.05} = 201.14$, $\chi^2_{0.001} = 233.80$.

†Kolmogorov–Smirnov test: $D_{0.05} = 0.0959$, $D_{0.01} = 0.1191$, $D_{0.001} = 0.1343$ on 172 d.f.

distribution[20] gave a very close fit, particularly when I_1 was omitted ($P > 0.05$), though its 'characteristic' slope was flatter. Features in common between expectations from the MacArthur model and trends in the heath vegetation are a homogeneous habitat, species related taxonomically (taxocenes) and structurally (synusiae), and populations stable and long-lived (no annuals were present)—all suggesting lack of dominance and strong competition for resources[5]. Points of disparity are the views that the model is only applicable to limited numbers of animal species in communities of limited extent[5].

By substituting number of individuals (I'_i) for I_i as the measure of importance it was possible to consider Fisher's logarithmic distribution[21]. This gave the closest fit of the four classical curves ($P > 0.05$), though its 'characteristic' slope was steeper. The model assumes that additional individuals encountered include previously uncollected (rare) species in preference to previously collected (common) species, highlighting, in this case, the large number of species of low relative importance. The model implies a spatially unlimited community, such that further sampling would have included progressively more species which, as already stated, would not have occurred in the present case. Even in the final category ($I'_i = 1$, $I_i = 0.00$–0.035) there were five rather than the 28 species predicted by the logarithmic function. Though lacking a theoretical basis at present, the best fit to the 'characteristic' slope of the I–V curve was provided by the truncated exponential distribution. Omission of the first and last 10% of species gave a near perfect fit (linear regression gives $r^2 = 0.992$), with implications for measures of diversity discussed below. The applicability of the total exponential curve was limited by its failure to change slope at the tails of the I–V curve, though the fit was still close ($r^2 = 0.971$).

The reason for the poor fit of the lognormal curve may be that the heath vegetation was not as rich as expected. But using Gleason's index, based on the species–area curve[22], the study site compared favourably with a tropical rain forest in Central Malaya (". . . the richest community ever recorded")[8] (Table 2). The figure for the rain forest was achieved with a much greater area (22.3 ha), far fewer individuals (2,629)—and hence a much higher Williams index[21], and almost at the opposite end of the life-form spectrum. The inverse of Simpson's index[23], based on the geometric function, and the Shannon–Wiener index[24], based on the severe restrictions of information theory and tending to 'downplay' differences in species-rich communities[5], had values in excess of a "species-rich" tropical forest in the Panama Canal Zone (". . . among the highest (H' values) ever reported for any community")[9]. The log-cycle equitability index[5], based on the geometric model, was more than twice that for a swamp-heath with "a remarkably high species richness" in south-eastern Queensland[10]. Whittaker's equitability index[4], based on the lognormal distribution, was more than four times that for the most diverse site along a 730–2,766 m gradient in south-eastern Arizona. Clearly, the present study site had 'surpassed' a lognormal relationship to reach a linear-sigmoid curve more related to the MacArthur or logarithmic models.

The restricted theoretical applicability of the usual measures of equitability described above, and the dissimilar slopes of the fitted theoretical distributions to that of the I–V curve under study, indicate the need for an index that more truly reflects the characteristic slope of the I–V curve and is not bound to particular community models. An inspection of 40 I–V curves in the literature[1,2,4,5,25,26] showed that, in addition to the present I–V curve, all sets of data would fit an exponential curve, especially if the first and last 10% of data were omitted. The remaining 80% have a slope (b) which can be determined from $I_r = a \exp(-b\ S_r)$. Then the characteristic slope equitability index (E_b) is given by

$$\Delta S/\Delta\log_{10}\hat{I} = (S_{90\%-1} - S_{10\%+1})/(\log_{10}\hat{p}_{10\%+1} - \log_{10}p_{90\%-1})$$

$$= 1/0.434b$$

(see Tables 1 and 2 for the meaning of the symbols).

The arguments for omitting the first and last 10% of the data are essentially that they describe slopes that may be quite different from the characteristic slope; their component species are unlikely to be characteristic of (restricted to) that community, and their slope generally shows little variation between communities fitting quite different I–V models. Indeed, a fuller description of the community might include the best fit lines of the three slopes, though this would seldom provide more information than E_b alone. Within the limits discussed here, the average concentration of importance for the first 10% of data ($\Sigma_1^{S_{10\%}} p_i^2/S_{10\%}$) may be informative for certain purposes. On the same basis, the last 10% of data should indicate the degree of rarity of the least common species in the community. But the presence/absence of species in the last 10% is very dependent on sample size, some ecologists even being reluctant to quantify at this level[26,27].

Table 2 Values of various indices of species diversity for the study site and other species-rich communities

Index	Formula	Study site	Previous highest	Reference
Gleason's	$\frac{S}{\log_{10}A}$	64.10 (Sector$_{10}$ = 69.2)	67.87	8
Williams	$\frac{S}{\log_e\left(1+\frac{I'}{\alpha}\right)}$	28.02 (Sector$_{10}$ = 37.4)	114.2	8
Simpson's (inverse)	$\frac{1}{\Sigma_1^S p_i^2}$	38.77	34.48	9
Shannon–Wiener	$-\Sigma_1^S p_i\log_2 p_i$	6.12	5.86	9
Log-cycle	$\frac{S}{\log_{10}p_1 - \log_{10}p_s}$	48.73	23.10	10
Whittaker's	$\frac{S}{4\sqrt{(\Sigma_1^S(\log_{10}p_i - \log_{10}\bar{p})^2/S)}}$	54.90	11.90	4
Characteristic slope	$\frac{S_{90\%-1} - S_{10\%+1}}{\log_{10}p_{10\%+1} - \log_{10}p_{90\%-1}}$	86.73	≃41.64	2

See Table 1 for meaning of symbols. In addition, A = area in m², $\log_{10}\bar{p} = \Sigma\log_{10}p_i/S$, $p_{10\%+1}$ = estimated p (or I) for the (10% + 1)th species from the truncated exponential curve fit in Table 1.

Using Whittaker's criteria of a good diversity index[5], E_b can be considered relatively independent of sample size—rare species do not influence the slope, and the effect of any overestimation of importance of dominants in small samples is minimised. Sampling error is low—80% of the species collected contribute to the index, standard regression analysis can be used to determine the level of sampling error and dispersion about the mean is minimised by removing the tails. E_b is conceptually meaningful—it is literally (the inverse of) the slope of the best fit line; the numerator is based on the total number of species and hence the formula also gives the relationship between richness and equitability. It is suitable for any taxocene or measure of importance, and its inverse-slope character enhances its sensitivity to changes in evenness.

E_b for the study site was more than twice that for a naturally regenerated deciduous forest in southern Illinois of "exceptionally high species diversity"[2]. Unlike the latter site, there was little evidence of vertical heterogeneity or creation of microhabitats in the heath vegetation that could explain its much greater diversity. Certainly the small life forms, open canopies and similar resource requirements would encourage close packing of these slow-growing perennials with minimal opportunity for dominance. This would be enhanced by proneness of the site to fire, drought and severe leaching. In addition, the site's location in a conjectured 'centre of diversification' of the south-western flora[28]—45% of the species belonged to a mere three families—would provide a large pool of coloniser species. Assuming that fluctuations about the present climate have occurred here in the past, this proposition is consistent with Stebbins' argument[29] that speciation is most favoured in transition zones between xeric and mesic or tropical environments.

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Reciprocal altruism in *Papio anubis*

ALTRUISM is behaviour that benefits another individual at some cost to the altruist, costs and benefits being measured in terms of individual fitness. 'Reciprocal altruism' (ref. 1) implies the exchange of altruistic acts between unrelated individuals as well as between relatives. If the benefits to the recipient of an altruistic act exceed the costs to the altruist, and if the recipient is likely to reciprocate at a later time, then the cumulative benefits for both individuals will have exceeded the cumulative costs of their altruism. Natural selection would favour individuals that engaged in reciprocal altruism if they distributed their altruism with respect to the altruistic tendencies of the recipient, preferring individuals that were most likely to reciprocate and excluding nonaltruists from the benefits of further altruism. This model has been difficult to test because it is usually impossible to be certain that an example of altruism is not the product of 'kin selection'[2]. The genetic relationships between individuals in animal populations are seldom known and reciprocal altruism can only be cited when it can be found to occur regularly between unrelated individuals. I report here that altruistic behaviour involving the formation of coalitions among male olive baboons (*Papio anubis*) fulfils the criteria for reciprocal altruism.

Eighteen adult male *P. anubis* in three troops at Gombe National Park, Tanzania were studied for more than 1,100 h, between May and December 1972 and from June 1974 to May 1975. All data were collected on a focal sample basis[3]. Focal individuals are referred to as 'targets'. Each animal was observed regularly for a fixed period. Observations were made on foot at 5–10 m from the animal. Baboon studies have been in progress since 1967 and data concerning blood relationships and dates of transfer between troops are available. All males leave their natal troop at Gombe, and the males who breed within a particular troop are those that have transferred into that troop from elsewhere[4]. Males known or thought to have transferred into their troop of residence are termed 'adult males'. Sexually mature males still residing in their natal troop are 'natal males'. During the study, there were three, six to eleven, and eight to eleven adult males in the respective study troops.

The effectiveness of temporary coalitions of adult male *Papio* spp. during aggressive interactions against a single opponent has been described in *P. anubis*[5] and in *P. ursinus*[6]. Encounters between coalitions and single opponents in *P. cynocephalus* did not seem to affect subsequent dyadic encounters between a coalition member and the opponent[7]. Ransom noted that coalitions of *P. anubis* at Gombe were generally formed by one male enlisting a partner to help fight against an opponent[8]. The partner had not been involved directly in the encounter with the opponent before his enlistment. Coalitions were sometimes formed in attempts to separate an opponent from an oestrous female. *P. anubis* form exclusive consort pairs lasting for up to several days. If the female became available after such an attempt, she could be taken by only one of the two coalition partners.

Attempts at enlisting a coalition partner are referred to as 'soliciting'—a triadic interaction in which one individual, the enlisting animal, repeatedly and rapidly turns his head from a second individual, the solicited animal, towards a third individual (opponent), while continuously threatening the third. The function of headturning by the enlisting animal is to incite the solicited animal into joining him in threatening the opponent. An 'occasion' of soliciting is a bout of the behaviour followed by a gap of more than 10 s. The distribution of the number of 'occasions' of soliciting to the same partner per observation period was tested against a cumulative binomial distribution and did not differ significantly from expected values. Therefore, 'occasions' of soliciting are considered to be statistically independent from each other. For every occasion that soliciting involved the target male during an observation period, the identity and actions (enlisting, solicited, or opponent) of each participant were recorded. 'Occasions' were not recorded consistently in 1972, so data from that period are not included in measures of frequency. Although coalitions occasionally formed spontaneously, only those that resulted from soliciting are considered.

There were 140 examples of one adult male soliciting another during the 1974–75 study period. Soliciting resulted in coalitions on 97 occasions. On 20 occasions the opponent was consorting with an oestrous female and adult males were more likely to join a coalition if the opponent was in consort (2 × 2 $\chi^2 = 5.91$, $P < 0.02$, $n = 140$). On six occasions during both study periods the formation of a coalition directed against a consorting male resulted in the loss of the female by the opponent. In all six cases the female ended up with the enlisting male of the coalition ($P = 0.032$, two-tailed, sign test); the solicited male generally continued to fight the opponent while the enlisting male took over the female. In each of those cases the solicited male risked injury from fighting the opponent while the enlisting male gained access to an oestrous female. In most other examples of soliciting, no resource appeared to be at stake. The greater willingness to join a coalition against a consorting male may be related to the greater benefits that the altruism bestows on the recipient in those cases.

Thirteen different pairs of males reciprocated in joining coalitions at each other's request on separate occasions. These pairs comprised 12 different males. In six of these pairs, both pair members successfully enlisted their partner against the same opponent. Individual males which most frequently gave aid were those which most frequently received aid. There was a strong correlation between the frequency with which adult males joined coalitions and the frequency with which they successfully enlisted coalition partners ($\hat{\rho} = 0.84$, $z = 2.87$, $P < 0.004$, Kraemer test[9] based on Spearman correlations).

Each male tended to request aid from an individual who in turn requested aid from him. Using only one occasion of soliciting per pair per observation period (since spatial patterns are relatively stable), the distribution of soliciting by each of the 18 target males was examined to find which other male each target solicited most often, that is, his 'favourite partner'. Only the ten targets who solicited other males on four or more occasions were included. After the favourite partner was found for each target male, the distribution of soliciting by each partner was similarly examined. For nine out of ten target males, the favourite partner in turn solicited the target male more often than the average number of occasions that the partner solicited all adult males in their troop ($P = 0.022$, two-tailed, sign test) (Table 1). These results suggest that preferences for particular partners may be partly based on reciprocation.

It is difficult to test whether a non-altruist is excluded from further altruistic exchanges; a refusal to join a coalition in any given instance may occur because the costs to the potential altruist on that occasion are particularly high. There is evidence, however, that adult males are more likely to join coalitions with individuals which in turn would be able to help them. Females and juveniles ('non-males') solicited adult males 12 times, but adult males never solicited 'non-males' ($P < 0.001$, two-tailed, sign test). Adult males were less likely to respond to the solicitings of 'non-males' than they were to other adult males (2 × 2 $\chi^2 = 7.76$, $P < 0.01$, $n = 152$), even though 'non-males' solicited adult males against other 'non-males' far more often than adult males solicited other adult males against 'non-males' (2 × 2 $\chi^2 = 43.76$, $P < 0.001$, $n = 110$). Adult male baboons are very much larger than either adult females or juveniles, so that even though the aid of an adult male to a 'non-male' would generally incur small costs to the adult male, the benefits to the male from having a 'non-male' partner would be trivial, since a 'non-male' could not provide effective help in an encounter against another adult male and an adult male would not need help in fighting a 'non-male'.

The adult males in the troops studied transferred into their troop of residence singly[10]. The origins of many males entering a study troop were not definitely known, so it was impossible to determine the precise degree of relatedness between most adult males. The previous troop and dates of transfer of each male were known, however. In 4 of the 13 reciprocating pairs, both partners were first observed as young adults in different troops when they probably had not yet transferred for the first time. They did not reside in the same troop for another 5 yr. One pair not included earlier comprised an adult male and a 'natal' male which were known to have been born in separate troops. Although there is no proof that any of these individuals are completely unrelated, it is unlikely that they are close relatives.

Table 1 Soliciting activity of favourite partners of target males

Target	No. of occasions FP solicited target	Mean no. of occasions FP solicited each male	FP solicited target more; + or less; − than average
BBB	3	2.7	+
CRS	3	1.2	+
DVD	0	1.0	−
EBN	5	1.0	+
GRN	1	0.7	+
JNH	2	1.1	+
LEO	4	2.7	+
MNT	1	0.4	+
WDY	5	2.7	+
WTH	4	2.8	+

FP, favourite partners.

In contests between a coalition and an opponent a previously uninvolved partner may benefit the enlisting male by reducing the latter's risk of injury in fighting the opponent. (Although only one male has been known to die from wounds received in a fight since 1967, non-fatal wounds are common.) By participating in a fight in which he would not have been otherwise involved, his aid will have been at some cost to himself. Whether or not the benefits to the recipient exceed the costs to the altruist (as required by the model) is difficult to determine. But, for the opponent, the potential costs in facing two males simultaneously rather than only one might be so much greater that it would often be to his advantage to avoid the coalition without fighting. If so, then the actual costs to the altruist are less than the reduction in costs to the enlisting male since the enlisting male would then be less likely actually to fight the opponent. When the formation of a coalition involves gaining access to an oestrous female further benefits are involved. Coalitions are the most common way of aggressively taking over an oestrous female from a consorting male at Gombe (D. A. Collins, in preparation). During the reproductive life of a male, which extends over 10 yr, there may be a large number of situations where it would be advantageous to enlist a coalition partner in an encounter against a consorting male. The number of offspring that a male sired as a result of participating in reciprocating coalitions would be greater than if he did not, while his lifespan would probably not be appreciably shortened by aiding coalition partners.

There are probably occasions when altruism is not involved. For example, the solicited male may sometimes join a coalition when there is a prospect of immediate benefit to himself. In such cases, explanations of the animal's behaviour are not necessary; the behaviour is not detrimental to his own fitness. The occurrence of genuine altruism, however, seems to be common enough to demand an alternative explanation.

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Sound as a host-detection cue for the soft tick *Ornithodoros concanensis*

Two parasitic flies are attracted to their invertebrate hosts by sound: a tachinid, *Euphasiopteryx ochracea*, is attracted to field crickets by their song[1] and a sarcophagid, *Colcondamyia auditrix*, orients acoustically to cicada song[2]. We now report that an acarine, the argasid tick *Ornithodoros concanensis*, makes use of the vocal sounds of a vertebrate host as a cue in its host-finding behaviour.

Recorded vocalisations of nestling cliff swallows and electronically generated sounds of specific frequencies were used to test responses of unfed and engorged nymphs of *Ornithodoros concanensis*. Specific frequencies and their pattern of modulation were selected for testing after sonographic analysis of the recorded nestling vocalisations. Dominant sounds produced in the calls were in the 3,000–8,000-Hz range. Three frequencies (3,000, 5,200 and 8,000 Hz) prevalent in the nestling vocalisations and additional sounds above (12,000 and 15,000 Hz) and below (500 and 1,000 Hz) these levels were selected for testing. During experiments the generated sounds were modulated to a pulse repetition rate of one per s and a duration of 140 ms to approximate the pattern of nestling vocal emissions. Sound intensities for all generated frequencies and the recorded nestling sounds graded down from 62–66 dB on the edge of the stage proximal to the speaker (8 cm from the speaker) to 61–64 dB at the centre (19 cm from the speaker) to 60–62 dB at the distal edge of the stage (30 cm from the speaker). Control experiments were done with the speaker turned on but without taped bird sounds or output from the oscillator. The stage consisted of a circular paper grid (22 cm diameter) on a piece of 2.5-cm thick styrofoam. The loud speaker was positioned 8 cm from the stage and its base rested on a 2-cm thick polyurethane foam pad. The stage and speaker were enclosed within a rectangular sound arena (69 cm long, 24 cm wide and 31 cm high) constructed of 10-cm thick porous polyurethane foam. A diffuse light centred 150 cm above the stage generated light of less than 0.22 mlm cm^{-2} on the stage surface. During trials the temperature on the stage was 26.6 ± 1 °C and the relative humidity ranged from 15 to 18%. Before use in an experiment, ticks in individual vials were maintained for at least 6 weeks after feeding at 85% relative humidity, 25 °C, and in a 24-h light cycle with 14 h of light. Trials were begun 2 h after the onset of darkness. To initiate a trial the vial containing a tick was inverted gently and the tick was allowed to fall on to the centre of the stage. Tick movements were observed and the point at which each tick left the circular stage was recorded on a data sheet. At the conclusion of a trial a data-set resembled one of the plots in Fig. 1. The mean number of ticks used in each trial was 22. The numbers tested at each frequency and in controls are shown in Table 1, together with a summary of statistical analysis. Rayleigh's test[3] was used statistically to evaluate whether the circular dispersion of the ticks was non-random.

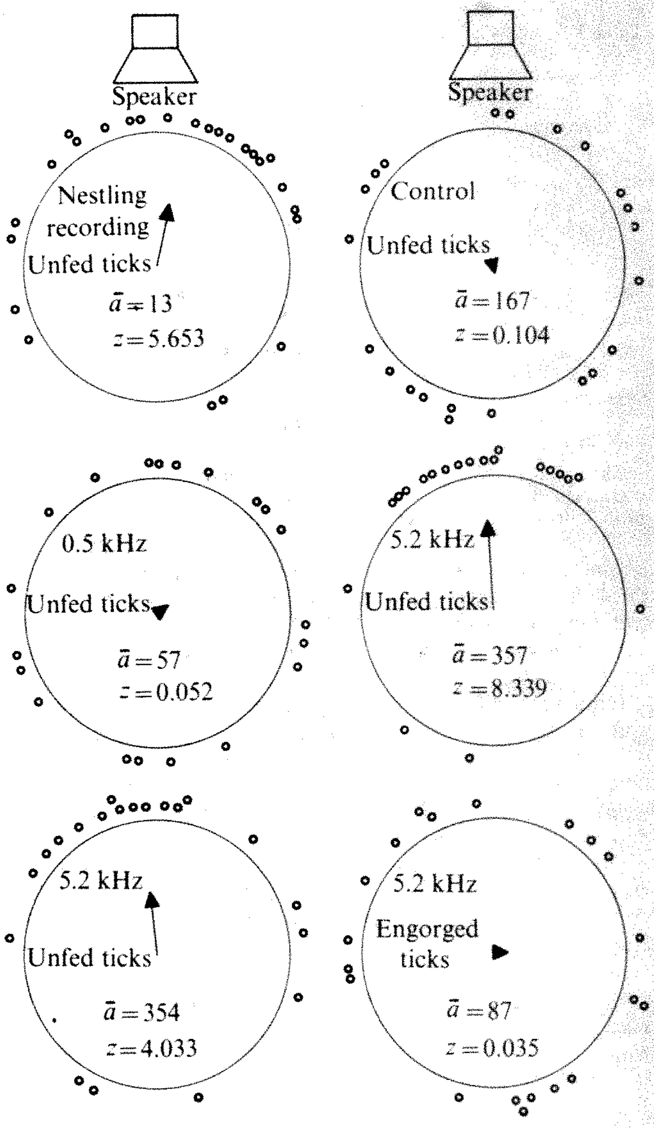

Fig. 1 Selected examples of the dispersal of ticks illustrate their tendency to move toward a speaker generating specific sounds. The small circles represent the positions to which ticks moved after being released in the centre of the circular stage. Symbols relating to the circular distribution analysis are: $\bar{a}$ = the mean angle vector; z = Rayleigh's critical value; and the length of the vector indicates the amount of relative circular dispersion of the group of ticks. Tape recordings of nestling cliff swallows were made with a Panasonic tape recorder (RQ-209-DAS) and a Panasonic dynamic microphone. The tape recordings were analysed with a Kay sonograph 6061A spectrum analyser. Specific sound frequencies were generated with a Hewlett–Packard Model 200 CDR wide range oscillator, a Grass S-5 stimulator, and a Grass AM-4 a.c. audio monitor. The Oxford loudspeaker (8.9-cm, 3.2 Ω) that was used has a frequency response of 50–15,000 Hz. Light intensity was measured by a Weston Model 76 illumination meter. Sound intensities were measured with a Type 2203 Brüel and Kjaer precision sound level meter.

In experimental trials unfed nymphs demonstrated a significantly pronounced movement toward the speaker when the recording of nestling sounds was played and when the frequencies within the nestling sound range (3,000, 5,200 and 8,000 Hz) were generated. Such movements were not seen with trials involving 500, 1,000, 12,000 and 15,000 Hz nor in control trials of unfed ticks (Table 1). After being released on the centre of the stage, unfed ticks characteristically would remain akinetic for 3–5 min. Positively responding ticks would then orient toward the sound source before proceeding forward with movements involving wavy or zigzag deviations from a straight line. From our results it was not possible to determine whether the pattern of orientation is a klinotaxis or a tropotaxis. A few ticks responded with pronounced turning suggestive of a klinokinesis. During trials involving the sounds to which ticks responded positively, it often seemed that the first pair of legs contracted in synchrony with each sound pulse from the speaker. By directing the modulated sounds of specific

Table 1 Analysis of tick responses to generated sound frequencies and recorded host vocalisations

Frequency (Hz)	Experimental trials No. of ticks	Experimental trials Probability	Controls No. of ticks	Controls Probability
Unfed				
500	21	NS	20	NS
1,000	19	NS	20	NS
3,000	23	$P < 0.001$	20	NS
5,200	20	$P < 0.001$	20	NS
8,000	30	$P < 0.05$	22	NS
12,000	21	NS	22	NS
15,000	20	NS	22	NS
Nestlings	25	$P < 0.005$	22	NS
Engorged				
5,200	20	NS	20	$P < 0.02$

The null hypothesis of a random distribution of ticks around the circle was accepted and the trial results were considered not significant (NS) if the probability was greater than the 0.05 level.

frequencies (for example, 3,000 Hz) at a tick being observed through a dissecting microscope, it was possible to confirm twitching or contracting of the first pair of legs with each sound pulse. This suggests a possible location of sound receptors in these appendages.

Immediately after observing a positive response of one group of unfed nymphs to 5,200 Hz, the ticks were fed on pigeons and individually retested at the same frequency. In contrast to the unfed ticks, engorged individuals were not temporarily akinetic when introduced to the stage, but immediately began to move. The pattern of dispersal of the engorged ticks was random.

Ornithodoros concanensis is a parasite of cliff swallows (*Petrochelidon pyrrhonota*), other birds and bats[4] associated with cliff and cave habitats in western North America. Concealed in the cracks and crevices during the day, the ticks move up the cliff face at night to obtain blood meals from cliff swallows roosting in their retort-shaped nests. Bird activity diminishes sharply at sunset but vocalisation in the nest continues during the night, particularly while young birds are present. Host-finding activity of unfed ticks begins 30 min to 1 h after sunset (approximately 2100 h), peaks 1–2 h later, and ebbs by 0100 or 0200 h. Experiments have indicated that nymphal *O. concanensis* are not stimulated by elevated concentrations of carbon dioxide that approximate normal (4–5%) or below normal levels of host-breath emission. Furthermore, host odour failed to elicit positive reactions and heat was important only at close range[5]. All indications are that sound is the principal cue used by *O. concanensis* for orientation to and location of host cliff swallows. After a blood meal is obtained the bird sounds are no longer attractive to the engorged tick and a combination of stimuli influences its movement out of the nest and back into a harbourage.

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Evidence for role of flagella as sensory transducers in mating of *Chlamydomonas reinhardi*

FLAGELLA and cilia are motility organelles of many cells. Modified cilia are present in most metazoan sensory cells[1], and mechanosensory transduction by cilia is found in many protozoans and some invertebrates[2]. The question arises as to whether flagella used primarily for motility also have sensory functions. In the unicellular alga *Chlamydomonas reinhardi*, flagella act as organs of motility. It has been observed, however, that flagellar contact initiates the mating process when gametes of opposite mating types are mixed[3–6]. We have been studying this phenomenon and report here observations on the role of flagella in initiating the mating reaction.

The mating process commences following the mixing of (+) and (−) gametes of *Chlamydomonas reinhardi*. Several physiological changes have been observed. These include cell wall lysis[4–6] and fertilisation tubule formation in the (+) gametes[5,6]. We have previously reported the accumulation of carbohydrates in a cell-free supernatant of mating cells, probably a result of cell wall lysis. The accumulation is specific and occurs only when live gametes of opposite mating types are mixed[7].

As shown in Fig. 1, there is a 45-min delay in carbohydrate accumulation if the gametes are deflagellated at the time of mixing. Flagellar regeneration in *Chlamydomonas reinhardi* has been well studied[8–11], but not in a mixture of

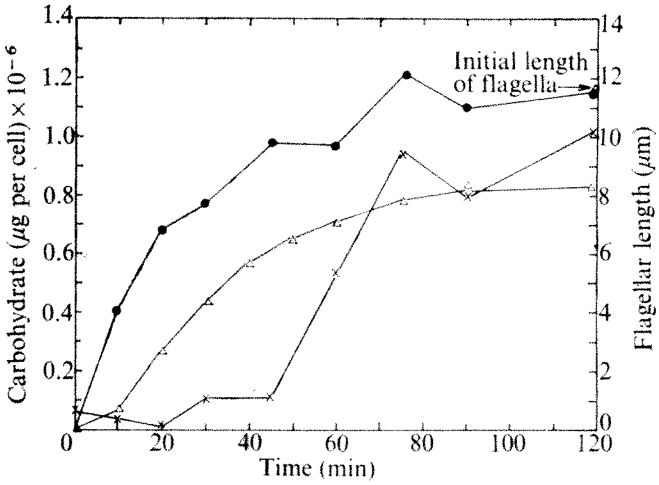

Fig. 1 Carbohydrate release and flagellar regeneration. *Chlamydomonas reinhardi* were grown as described before[7], except that they were on a 12-h light–12-h dark cycle. At time 0, active (+) and (−) gametes were mixed. The flagella of the experimental group were detached upon mixing using the *p*H method[12]. Deflagellation was accomplished by lowering the *p*H to 4.5 for 1 min with 10% acetic acid and neutralising with KOH. Detachment was followed with phase microscopy and more than 99% of the cells became deflagellated. Flagellar regeneration was observed in at least 90% of the cells which were deflagellated. The non-deflagellated control group had acetic acid and KOH added simultaneously. Samples of the cell suspension (1.6×10^7 cells) were taken at intervals and carbohydrate analysed in the cell-free supernatant as described before[7]. Flagellar regeneration was followed using the methods of Rosenbaum *et al.*[9]. It is interesting to note that after 2 h of regeneration, the flagella had reached only about 75% of their original length. For vegetative cells it has been reported that the flagella are 90% regenerated after 60 min[9]. We attribute the discrepancy to the fact that in our case, the cells are mating, and that during the actual cell fusion flagella grow slowly or not at all. ●——●, (+) and (−) gametes, non-deflagellated control; ×——×, (+) and (−) gametes, flagella detached upon mixing; △——△, flagella length in μm. Each point represents the average of two experiments.

mating gametes. To ascertain the relationship between flagellar length and initiation of mating, we monitored flagellar regeneration in a population of deflagellated mixed gametes. As indicated by the carbohydrate accumulation (Fig. 1), the mating reaction commences after the flagella have regenerated to more than 50% of their original length. Cells regain motility 25 min after deflagellation, and at this point the flagella are about 25% of their original length. There is, therefore, a delay of at least 20 min after the return of motility before carbohydrate accumulation can be detected. Thus, it seems that the limiting factor for mating is the length of the flagella, and that some sort of flagellar differentiation occurs when the organelles are about 50% regenerated. This maturation allows the organelle to act as a sensory transducer for the mating reaction.

An alternative explanation involves the lowered probabilities of gametic interaction when flagella are shorter than normal. This explanation is unlikely, however, for two reasons. First, the cells are at a very high concentration (2×10^7 cells ml^{-1}). Second, the 45-min delay in mating, as measured by carbohydrate accumulation, is present even if only one of the mating types is deflagellated and mixed with the opposite mating type. This implies that in spite of the increased likelihood of encounter resulting from the presence of full length flagella in one of the mating types, mating did not occur until the flagella from the deflagellated cells attained 50% of their normal length. The crucial finding is that the time delay in this situation was identical to the delay following deflagellation of both mating types.

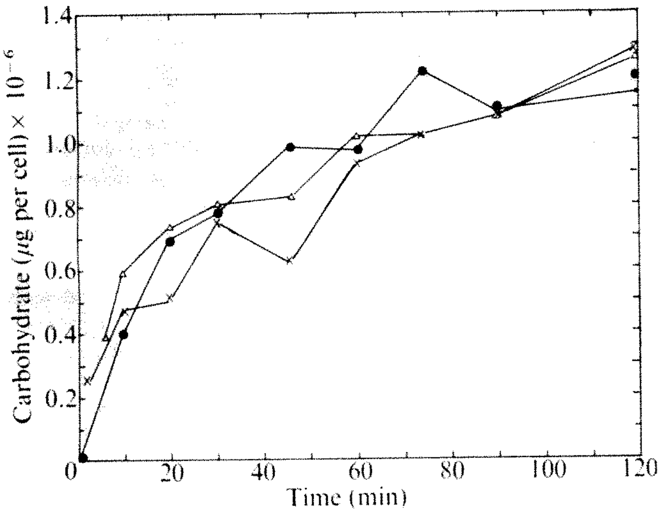

Fig. 2 Carbohydrate release and detachment of flagella. Conditions of cell growth, flagellum detachment and carbohydrate analysis are the same as in Fig. 1. At time 0, active (+) and (−) gametes of *Chlamydomonas reinhardi* were mixed. At 2 min and 4 min after mixing, aliquots were taken and deflagellated. At intervals thereafter, samples of the cellular suspension (1.6×10^7 cells) were taken and analysed for carbohydrate as previously described[7]. ●——●, (+) and (−) gametes, non-deflagellated control; ×——×, (+) and (−) gametes, flagella detached 2 min after mixing; △——△, (+) and (−) gametes, flagella detached 4 min after mixing. Each point represents the average of two experiments.

Figure 2 shows the effect of deflagellation at various times after mixing the gametes. It can be seen that cells deflagellated at either 2 min or 4 min after mixing continue in the normal time course of carbohydrates accumulation. Since the deflagellation procedure (a quick *p*H change—see Fig. 1) does not in itself induce the gametes, the lack of a delay in carbohydrate accumulation indicates that the flagella act as transducers within the first 2 min of mating.

From our data we can conclude that a signal responsible for initiating the mating process in the gametes of *Chlamydomonas reinhardi* is transmitted by means of the flagella. Without flagella that induction is delayed for 45 min, when the flagella have regenerated to more than one half their original length. This form of sensory transduction occurs within the first 2 min of mating, since the loss of flagella after that point does not inhibit the mating reaction as measured by carbohydrate accumulation. The reaction seems to be associated with the 'mature' tips of the flagella, and because of its specificity we assume it to be chemical. At this point, however, we cannot be specific as to the actual mechanism of the reception and transduction of the mating signal. The significance of this work lies in the fact that we have been able to demonstrate that the flagella of *Chlamydomonas reinhardi* are both organelles of motility and sensory transducers in the mating reaction.

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Defective polymorphonuclear leukocyte function in a human periodontal disease

HUMAN periodontal diseases are the major cause of adult tooth loss. One such disease, localised idiopathic juvenile periodontitis (IJP) affects young people and is unusually severe, with a rapid progression. Previous studies have shed little light on the unusual susceptibility of some patients to this rare condition. Similarly, destructive periodontal syndromes have been described in patients with polymorphonuclear leukocyte (PMNL) dysfunction. We have measured two parameters of neutrophil function, chemotaxis and phagocytosis, and found that patients with IJP have significantly decreased PMNL function compared with healthy control subjects, but monocyte function remains unaffected in IJP. Reduced PMNL functions may contribute to the unusual susceptibility of patients to IJP.

Peridontal diseases are progressively destructive inflammatory processes affecting the supporting tissues of the teeth. By age 45 yr almost all individuals have periodontal disease; under age 45, a smaller but substantial part of the population is affected[1]. As periodontal diseases are accessible to study, they are excellent models for the investigation of host–parasite interactions in bacterial inflammatory disease.

Studies of IJP by many investigators have failed to establish any specific etiology[2] or account for the unusual susceptibility of certain individuals. A specific alteration in the host's protective response could explain the rapidly destructive character of this disease, but no major systemic involvement in such patients has been observed regularly. Therefore, any impairment in the host's protective response is likely to be subtle. Study of parameters of host resistance in patients with IJP, by sensitive procedures, may reveal mild alterations in protective response.

PMNL have been implicated in the inflammatory lesions of periodontal disease[3,4]. Patients with PMNL disorders such as cyclic neutropenia[5], agranulocytosis[6], and Chediak–

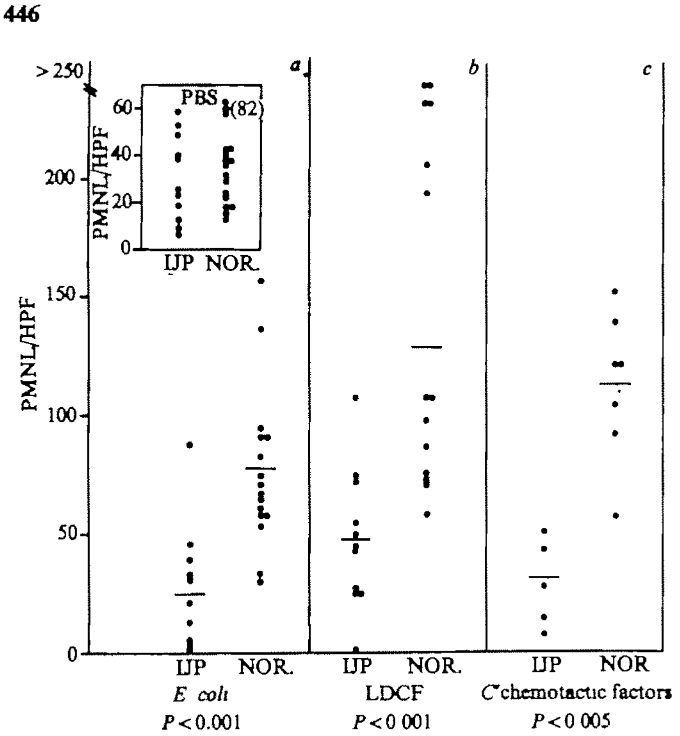

Fig. 1 Chemotactic response of PMN leukocytes (PMNL) from individuals with IJP compared to normal (NOR.) individuals. *a*, *E coli* 0127 was grown in trypticase soy broth for 18 h at 37 °C. The bacteria were removed from suspension by centrifugation and the culture filtrate was passed through a 0 20-µm filter. *b*, Leukocyte-derived chemotactic factor (LDCF) was prepared from a 48-h culture supernatant fluid of phytohemagglutinin-stimulated human peripheral blood mononuclear leukocytes. *c*, Chemotactic components of complement[18] were prepared by incubation (for 30 min at 37 °C) of 0 1 ml of fresh human serum with 0.9 ml of gelatin veronal buffer and 0.1 ml of a 1-mg-ml^{-1} suspension of lipopolysaccharide derived from *Salmonella typhimurium* (Difco). Each chemotactic preparation was divided into aliquots, used fresh or stored at −76 °C until used The chemotaxis assay was as described by Snyderman *et al*[12]. On any one day, responder cells prepared from peripheral blood of at least one individual with IJP were compared with responder cells prepared from one or more normal, age-matched subjects Leukocytes for each experiment were separated from erythrocytes by dextran sedimentation of whole blood Mononuclear cells and neutrophils were then purified from the leukocyte-rich plasma by Ficoll–Hypaque gradient centrifugation The neutrophils in the pellet were washed twice in 0.15 M NaCl buffered with 0.02 M sodium phosphate at *p*H 7 2 (PBS), and diluted with Gey's balanced salt solution (*p*H 7 2) to 2 5 × 10^6 cells per ml (personal communication from L. C. Altman). The neutrophils were placed in the top compartments and the chemotactic preparations or PBS (non-chemotactic negative control) were placed in the bottom compartments of modified Boyden chambers. The compartments were separated by a polycarbonate filter with 3-µm diameter pores (Nuclepore, Wallabs) The chambers were incubated for 30 min at 37 °C in an atmosphere of 5% CO_2 in humid air. The filters were then removed, stained, mounted on slides and viewed in a microscope. Cells migrating to the lower surface of the filter in 20 oil immersion fields were counted. Cell migration was expressed as the mean number of migrated cells per high-power field (HPF) for quadruplicate samples minus the mean background number of cells per field migrating towards a non-chemotactic (PBS) control. Data from IJP and normal subjects were grouped and compared by the Mann–Whitney U test Horizontal bars represent the arithmetic mean for each group

Higashi disease[7] show unusually severe loss of the supporting alveolar bone of the teeth. This may result from defective PMNL defence against oral bacteria. *In vitro* assays have shown leukocyte function to be reduced in many diseases. For example, Snyderman[8] has shown a defect in mononuclear leukocyte chemotaxis in patients with chronic mucocutaneous candidiasis. Defective PMNL chemotaxis is also associated with many disease conditions[9,10]. Gillman *et al.* reported a positive correlation between reduced neutrophil phagocytosis and susceptibility to middle ear infections in otherwise healthy individuals[11].

The subjects of our study were four males and five females in whom IJP was diagnosed as defined by the criteria of Kaslick[4]; three of the females were sisters. Diagnostic criteria, established by clinical and radiographic evaluation, included severe localised alveolar bone loss around the first molars and incisors of both maxillary and mandibular permanent teeth. Patients were 16–31 yr old (average 20.0 yr). Health status was established by a thorough medical history, a physical examination, and a battery of laboratory tests The results of the tests were always within established norms, and peripheral blood PMNL numbers were not different from those of a panel of normal subjects. Leukocyte differential counts were also within the normal range. All patients had a negative history of frequent or unusual illnesses and seemed in good general health.

Chemotaxis of PMNL and monocytes, and phagocytosis by PMNL were measured. Peripheral blood leukocytes were assayed simultaneously from patients with IJP and from normal subjects of similar age, sex and race. Samples from those with IJP were assayed on at least two separate occasions to demonstrate reproducibility of the observations. Chemotaxis was assayed using the modified Boyden chamber technique described by Snyderman[12], and phagocytosis was measured by the procedure of van Oss and Stinson[13].

When tested with *Escherichia coli* culture filtrate, leukocyte-derived chemotactic factor, and human complement-derived chemotactic factors[12], the polymorphonuclear leukocyte chemotactic response of patients with IJP was significantly less ($P<0.005$) than that of normal subjects (Fig. 1) By contrast, the chemotactic response of peripheral blood monocytes of IJP patients did not differ from that of the normal subjects No differences were seen between the groups in background PMN or monocyte cell migration against the non-chemotactant saline control.

Phagocytosis of opsonised *Staphylococcus aureus* by PMNL from patients with IJP and normal subjects was then compared (Table 1) All the subjects with IJP had depressed PMN phagocytosis as compared with normal controls. The contact angles formed by sessile drops of saline water on monolayers of PMNL (see refs 14 and 15) of patients and normal controls are also compared in Table 1 A higher contact angle, and thus a less hydrophilic surface of phagocytic cells is invariably accompanied by a decrease in phagocytic activity[15]. The differences in phagocytosis as well as in contact angles are significant at the 95% confidence level or greater with one exception. Leukocytes of two young patients with generalised periodontitis, clinically distinct from IJP, showed normal responses in assays for chemotaxis and phagocytosis

Thus there is a reduction in both chemotaxis and phagocytosis by PMN leukocytes in patients with IJP, although monocyte chemotaxis is normal In assays of the peripheral blood leukocyte blastogenic responses to phytohaemagglutinin and streptolysin 0 in these IJP patients, no obvious defects were found. More discriminating tests are in progress to assess cellular immunity more fully.

Lavine *et al.*[16] studied the response of PMNL from patients with early onset periodontitis to *E coli*-derived chemotactic factor, and concluded that PMNL from these patients are characterised by an intracellular defect in chemotaxis unrelated to serum inhibitory factors This disease entity was probably IJP, providing further evidence that such PMNL have defective chemotaxis.

Newman *et al.*[17] noted several distinct anaerobic Gram-negative rods, exhibiting surface translocation which seemed

Table 1 Phagocytic activities and contact angles of neutrophils of patients with IJP and of normal controls

	IJP Phagocytic activity*	Contact angle ± s.e.		Normal controls Phagocytic activity*	Contact angle ± s.e.
LB	5.5 ± 1.2	19.1 ± 0.3	CD	9.6 ± 2.3	17.7 ± 0.2
VB	4.4 ± 1.4	19.6 ± 0.5			
GB	4.2 ± 0.5	19.4 ± 0.5	MI	8.3 ± 1.0	17.8 ± 0.3
HO	7.5 ± 0.5	19.6 ± 0.5	BR	11.7 ± 2.1	18.1 ± 0.4
VA	2.9 ± 0.6	18.9 ± 0.5	LP	10.1 ± 1.2	18.3 ± 0.3
HU	4.8 ± 0.6	19.4 ± 0.4	BL	9.1 ± 1.3	18.3 ± 0.3
AD	3.6 ± 0.4	20.0 ± 0.4			
YO	4.6 ± 0.5	20.4 ± 0.4	CI	9.3 ± 0.7	17.6 ± 0.2
FI	6.1 ± 0.7	19.2 ± 0.7			
FI	5.1 ± 0.6	19.4 ± 0.4	PE	10.0 ± 0.9	17.5 ± 0.1

*Average number of microorganisms (*Staphylococcus aureus*, opsonised with 1% human γ-globulin) phagocytised per granulocyte, ± s.e. All tests on patients and on normal controls that were done on the same day are displayed horizontally. The differences in phagocytic activities between patients and normal controls are all significant to $P < 0.05$, except for LB ($P = 0.11$). The differences between contact angles measured on patients' and normal controls' granulocytes are all significant to $P < 0.05$.

to be present in the lesions of IJP. Rats monoinfected with these organisms rapidly develop severe alveolar bone loss, so these bacteria may be potential pathogens. They may release factors that penetrate the ulcerated gingival epithelium and enter the systemic circulation. Such factors may affect PMNL, resulting in altered functions such as chemotaxis and phagocytosis. Studies are under way to test this hypothesis. Alternatively, pre-existing impairment in PMNL function may exist in persons with IJP, and proliferation of these unique organisms may result from this diminished host response.

We have demonstrated that a reduction in PMNL phagocytosis and chemotaxis is common to patients with IJP. Whether this reduction in host protective response is pre-existing or a result of the disease remains to be determined.

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Allotypes of mouse IgM immunoglobulin

GENETIC polymorphism of the structural genes encoding the class-specific (heavy) polypeptide chains of the immunoglobulin (Ig) molecules provides a useful set of markers for elucidating the arrangement and expression of these genes. On the basis of various antigenic, physiochemical and biological properties, the immunoglobulins of the mouse have been divided into eight distinct classes, IgM, IgD, IgA, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgE, with a specific structural gene determining the heavy chain (H) for each class. Previous studies have documented the existence of a genetic polymorphism for five of these H-chain genes[1-3], with all the genes being closely linked constituting a heavy chain chromosome region. The most recent of these loci to be demonstrated (Ig-5, determining the δ chain)[3] was detected by the reaction of alloantisera prepared against spleen cells from mice differing in both *H–2* and allotype. These sera reacted with B lymphocytes of the appropriate allotype congenic strains, as assessed by either immunofluorescence, or cell surface iodination and polyacrylamide gel electrophoresis (PAGE). As most B lymphocytes express cell surface IgM as well as IgD (refs 4–6), we have further pursued these various approaches to identify Ig allotypes and have found a polymorphism of the heavy chain (μ) of murine IgM, a molecule found in pentameric form (19S) in serum, and in monomeric (8S) form on the surface of lymphocytes. This defines a locus, Ig-6, which encodes the μ chain of most mouse IgM immunoglobulin.

Alloantisera containing anti-IgM antibodies were raised in various strain combinations by two different methods. A small proportion of anti-allotype sera raised by repeated immunisation with pertussis anti-pertussis complexes[1], were found to contain antibodies to allotypic determinants on IgM molecules. However, the most effective antisera against IgM allotype have been raised by spleen cell immunisation (4–6 weekly injections of 10^7 spleen cells, with or without adjuvants) between strain combinations which differ in *H–2* type as well as allotype.

The binding capacities of these antisera against purified mouse immunoglobulins were assessed by radioimmunoassay[1] using purified ^{125}I-labelled mouse myeloma proteins[2]. Each labelled myeloma protein was more than 80% precipitable by the appropriate heterologous anti-mouse heavy-chain serum, while less than 10% was precipitated by all other anti-H-chain sera. The IgM proteins used were HPC-76 and MOPC104 (IG-6a) of BALB/c origin, and C.BPC112 (IG-6b) of C.B/20 (BALB/c Ig^b) origin (kindly provided by Dr M. Potter). The allelic designations for Ig-6

Table 1 Anti-Ig-6 antibody detected by radioimmunoassay, immunofluorescence and cell surface radioiodination/polyacrylamide gel electrophoresis (PAGE)

Alloantiserum		Precipitation of ^{125}I IgM† HPC-76 (Ig-6a)	Precipitation of ^{125}I IgM† C.BPC112 (Ig-6b)	Positive immunofluorescence‡ Strain	Positive immunofluorescence‡ Splenic B cells	PAGE analysis‖ Strain	PAGE analysis‖ Heavy chain detected
GA20:	C57BL anti-CBA* serum	Yes	No	BALB/c	Yes§	BALB/c	μ
				BALB/c.Ig^b	No§		
GA26:	C57 anti-CBA serum	No	No	BALB/c	No	BALB/c	—
MA3:	C57BL anti-CBA spleen	Yes	No	C57.Ig^a	Yes	CBA	μ,δ
				C57BL	No		
MB1:	SJA anti-BALB/c.Ig^b spleen	No	Yes	SJL	Yes	C57BL	μ,δ
				SJA	No		
MB2:	SJA anti-BALB/c.Ig^b spleen	No	No	SJL	Yes	C57BL	δ
				SJA	No		
MB3:	SJA anti-CWB spleen	No	Yes	SJL	Yes	C57BL	μ
				SJA	No		

*CBA anti-pertussis serum/pertussis complex.
†Determined by radioimmunoassay as previously described[1].
‡Revealed by indirect immunofluorescence using a fluoresceinated goat anti-mouse IgG (refs 3 and 9).
§Analysis was by visual fluorescence microscopy and/or with a fluorescence-activated cell sorter (FACS).
Yes: almost all B cells were stained. No: few, if any, B cells were stained.
‖PAGE analysis of ^{125}I-labelled membrane protein precipitated by antisera[3].
Mouse strains used in these experiments are of the following allotypes: Ig^a-CBA, BALB/c, SJA, C57.Ig^a; Ig^b-C57BL/6, BAB/c.Ig^b (BAB/14 or C.B/20), SJL, CWB (C3H.SW.Ig^b).

are assigned as for other allotype loci according to the Ig-1 allele of the type strains in which they are found[2]. Thus, Ig-6a is from BALB/c and Ig-6b is from C57BL/6. The precipitating activity of such antisera against ^{125}I-labelled HPC-76 is shown in Fig. 1. Antisera GA20 and GA26 were both made in C57BL/6 (C57) against pertussis anti-pertussis (of CBA origin) complexes. Each contained anti-Ig-1a antibodies (Fig. 1*b*), but serum GA20 also contained anti-Ig-6a antibody (Fig. 1*a*). In contrast, the serum MA3 prepared in C57 mice against CBA spleen cells, contained no anti-Ig-1a antibody, but did contain anti-Ig-6a antibody (Fig. 1*a* and *b*).

The anti Ig-6a sera were specific since they did not precipitate an IgM myeloma of C.B/20 (Ig^b) origin, C.BPC112 (see Table 1). Their specificity was confirmed by inhibition studies in which no myeloma protein of other classes (IgA, IgG$_1$, IgG$_2$) inhibited precipitation of ^{125}I-labelled HPC-76. Furthermore, unlabelled HPC-76, MOPC104 and normal serum from BALB/c did inhibit this precipitation, although MOPC104 was considerably less effective in inhibiting than the other inhibitors.

Antisera to another IgM allotype, Ig-6a, were prepared in SJA (Ig^a) against spleen cells of Ig-1b positive strains BALB/c.Ig^b (BAB/14) or CWB (C3H.SW.Ig^b). These sera precipitated C.BPC112 but precipitated neither HPC-76 nor MOPC104 (Table 1). Inhibitions of precipitation were found with Ig-6b containing sera and not with Ig-6a containing sera or myelomas. Similarly, no non-IgM myeloma protein inhibited ^{125}I-C.BPC112 precipitation with these alloantisera (data not presented).

Close linkage of Ig-6 to the Ig heavy chain region is implied in the use of allotype congenic strains for antiserum testing. Anti-Ig-6a (for example, antiserum MA-3 in Fig. 1*c*) is inhibited in its precipitation of HPC-76 by serum from BALB/c (Ig^a) and not by serum from the congenic mice BALB/c.Ig^b (BAB/14) (Fig. 1*c*) nor from C.B/20 (data not shown) which have all the alleles of BALB/c except these closely linked to Ig-1. Since something more than 21 backcross generations are involved in the derivation of these strains (they are not completely independently derived), very close linkage is indicated for Ig-6 to Ig-1 and the other heavy-chain loci[2].

These results with radioimmunoassay analysis demonstrate that antisera prepared against either cell-surface 8S IgM or serum 19S IgM, are capable of binding to 19S IgM molecules. We have also shown by immunofluorescence, that these antisera can react with cell-surface 8S IgM. Spleen cells from 8-week-old BALB/c (Ig^a), BALB/c.Ig^b, SJL (Ig^b), or SJL.Ig^a (SJA) allotype congenic strains were treated with a 5–10% (v/v) concentration of the alloantisera, washed and then reacted with a fluorescein-conjugated goat anti-mouse γ_1 and γ_2 antiserum. The latter serum was

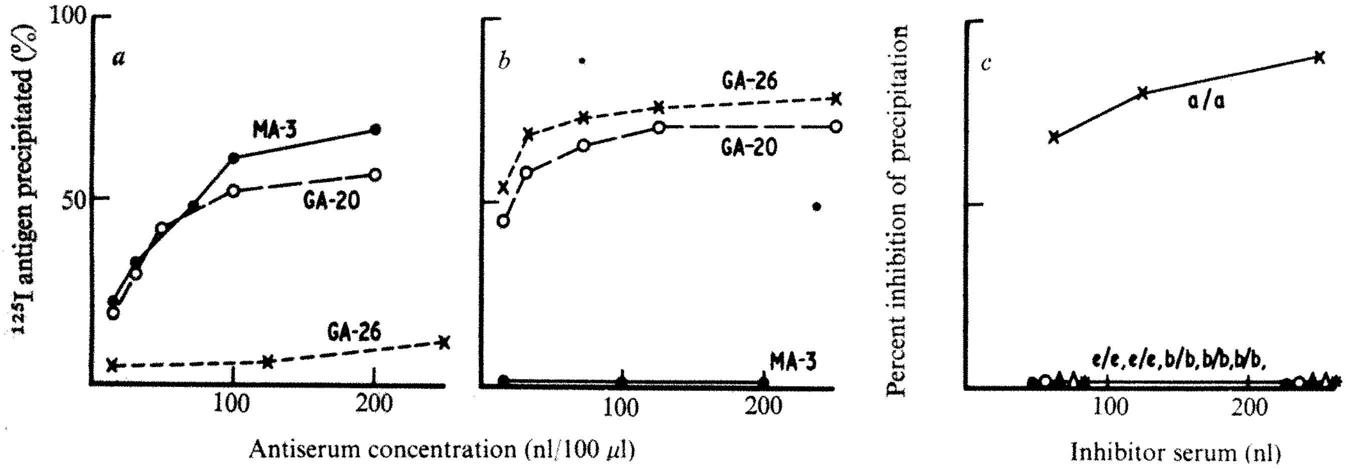

Fig. 1 Radioimmunoassay analysis of anti-Ig-6a antisera. The ability of alloantisera GA20, GA26 and MA3 to precipitate ^{125}I-labelled IgM (HPC-76 macroglobulin) or IgG$_{2a}$ (HPC-149) of BALB/c origin is shown in *a* and *b*, respectively, as the per cent precipitation of the labelled antigen against the amount of antiserum used. For panel *c* cell assays were performed in a volume of 100 μl with 5 ng of ^{125}I-labelled HPC-76 (Ig-6a). All inhibitor sera derived from 8-week-old mice of the following allotype congenic strains: BALB/c (Ig^a), BALB/c.Ig^b; C57BL(Ig^b), C57BL.Ig^e NZB (Ig^e), NZB.Ig^b. Note that the lack of inhibition by Ig^e sera indicates that Ig-6^a and Ig-6^e do not cross react with this antiserum. Further details of antigenic specificity will be published.

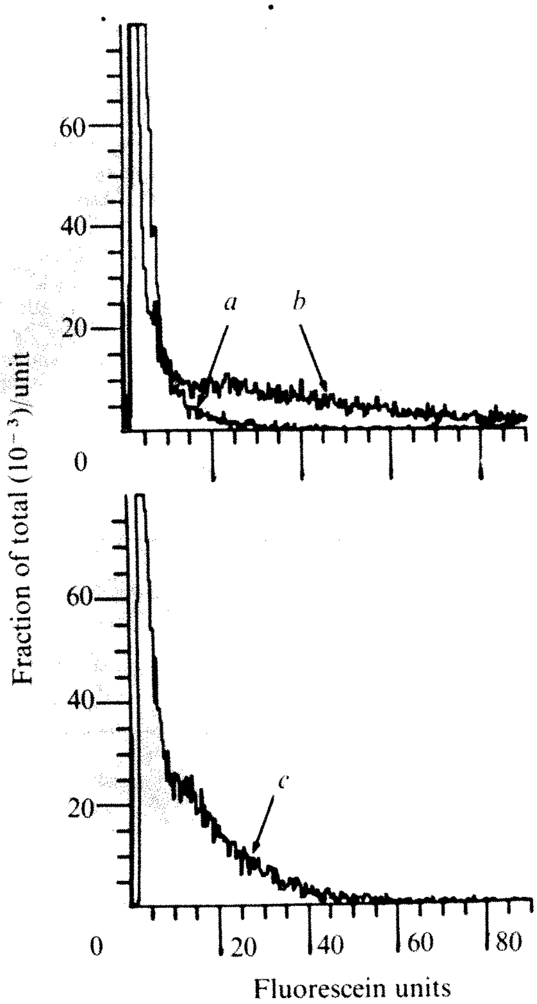

Fig. 2 FACS fluorescence analysis of anti-Ig.6^{b} serum. Fluorescence profile of SJA(Ig^{a}) anti-BALB/c.Ig^{b} serum on (*a*) SJA spleen cells (Ig^{a}), (*b*) SJL spleen cells (Ig^{b}), (*c*) SJL spleen cells after adsorption of serum with Sepharose-bound IgM myeloma C.BPC112 (Ig-6b). Cells (2×10^{6}) were reacted with 2 μl (diluted to 40 μl with buffered saline containing 0.2% sodium azide) SJA anti-BALB/c.Ig^{b} serum for 20 min at room temperature. These cells were washed and stained with fluoresceinated goat anti-mouse IgG for a further 20 min at room temperature and analysed on the FACS. Adsorption of this anti-Ig-6b serum by Sepharose-bound Ig-6a did not reduce its staining activity for SJL spleen cells.

completely non-reactive to IgM, as shown by immunofluorescence and radioimmunoassay. Fluorescence was detected by fluorescence microscopy as previously described[3], or by flow fluorometry with a fluorescence-activated cell sorter (FACS)[9]. These data are summarised in Table 1.

Table 1 shows that the same sera which precipitate secreted IgM also bind to cell surface IgM. Antisera made against pertussis–anti-pertussis complexes (that is, against secreted IgM) could be tested on spleen cells of any strain whereas antisera made against spleen cells could not because of the possible presence of antibodies to other cell surface alloantigens. With these latter antisera, spleen cells from mice congenic but for allotype with the antibody produced were used.

The antisera made in C57(Ig^{b}) against CBA(Ig^{a}) stained C57.Ig^{a} spleen cells and, of course, did not stain C57 cells (Table 1). Similarly, antisera made in SJA(Ig^{a}) against Ig^{b} cells stained SJL(Ig^{b}) but not SJA cells (Table 1 and Fig 2, *a* and *b*). Thus the surface molecules detected by these antisera are coded by genes in the Ig heavy-chain chromosome region.

The immunoglobulin class of the cell-surface molecules detected in these immunofluorescent reactions was determined by adsorption and blocking studies as well as by gel electrophoresis (PAGE). Serum MB1, SJA anti-BALB/c.Ig^{b} spleen, passed through a column of Sepharose-conjugated C.BPC112, an IgM of *b* allotype (Ig-6b), lost most of its staining activity for SJL (Fig. 2*c*). Therefore most of the reactivity is directed towards IgM. The antithetical serum against Ig-6a was similarly adsorbed by Sepharose MOPC104, an IgM myeloma from the Ig^{a} strain BALB/c (data not shown). Neither conjugate removed any staining activity from the opposite antisera. Heterologous anti-mouse μ chain blocked staining by both these antisera as well as by serum GA20. These same allotype antisera also precipitate cell surface IgM shown by PAGE of radioiodinated membrane proteins[3,7]. Thus, these alloantisera detect the allotypes Ig-6a and Ig-6b on cell-surface IgM.

Some antisera prepared by allogeneic spleen cell injections also have antibodies against IgD heavy-chain allotypes (Ig-5)[3,8]. Serum MB2 stained SJL spleen cells, although it contained no anti-Ig-6b reactivity as shown by radioimmune precipitation. This staining activity of SJL cells by serum MB2 was not removed by adsorption with insolubilised C.BPC112 nor was it blocked by pretreatment with heterologous anti-mouse μ. MB2 (Table 1) also precipitates radioiodinated cell surface IgD demonstrated by PAGE (ref. 3). Anti-Ig-5b activity also accounts for the small residual staining of IgM-adsorbed serum MB1 (Fig. 2*c*). The μ and δ precipitation activity of each of the sera detected by PAGE is given in the last column of Table 1. These data show that sera made against allogeneic spleen cells may have anti-allotype antibodies to δ chains (Ig-5), μ chains (Ig-6) or both in varying amounts. These new allotypes will serve as useful markers in following B-cell differentiation and gene expression. They may also aid in fine structure mapping of the Ig heavy-chain region which contains genes for both the constant and variable parts of the polypeptides. Some of these data has been presented[9]. A more detailed immunogenetic analysis of these loci will be published elsewhere.

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Specific glucocorticoid inhibition of growth promoting effects of prostaglandin F$_{2\alpha}$ on 3T3 cells

GROWTH and differentiation of mammalian cells is considered to be controlled in part by the interaction of various hormones and other growth promoting factors[1]. Corticosteroids are hormones with a wide range of tissue specificity, intracellular receptor proteins having been described in various tissues[2] and cultured cells[3,4]. Glucocorticoids exert paradoxical effects on cell proliferation. Thus they promote cell growth of the mammary gland epithelium in mice and rats[5,6] and in mouse and rat organ[7–9] and rat tissue culture systems[10], and DNA synthesis in cultured parenchymal rat liver cells[11]. But they inhibit all stages of the inflammatory response, including the reparative growth of fibroblasts, loss of immune competence due to damage to lymphocytes, lipolysis and other anabolic (contra-insulin) effects in adipose tissue[12]. In cultured fibroblasts hydrocortisone can, in different conditions, either stimulate[13] or inhibit[14–16] DNA synthesis and cell devision. The growth promoting effects of the corticosteroids in fibroblasts and mammary epithelial cells may be related to their ability to synergise with the fibroblast growth factor (FGF) (refs 17 and 18) and prolactin[10] respectively. Growth inhibitory effects may be due to the predominantly catabolic action of the glucocorticoids[12]. Here we report that hydrocortisone can inhibit specifically the stimulation of DNA synthesis and cell division induced by prostaglandin F$_{2\alpha}$ (ref. 19) in Swiss mouse 3T3 and 3T6 cells. We suggest that the cell-growth-regulating properties of the corticosteroids are in part dependent on the identity of the co-inducing growth controlling factors, and that glucocorticoid inhibition of prostaglandin-stimulated cell growth may have a role in the anti-inflammatory response.

Prostaglandin F$_{2\alpha}$ added at 500 ng ml^{-1} to relatively quiescent 3T3 cells stimulated 20% of the cells to synthesise DNA in 26 h, whereas without any additions only 0.3% of the cells were synthesising DNA (Fig. 1). Addition of increasing concentrations of hydrocortisone with PGF$_{2\alpha}$ caused a reduction in the fraction of cells synthesising DNA, which reached a maximum of 85% inhibition at a concentration of 20 ng ml^{-1} (Fig. 1 insert). Addition of insulin (50 ng ml^{-1}) alone had little effect, but when added with PGF$_{2\alpha}$ it increased the fraction of cells synthesising DNA, thus showing a synergistic effect. The addition of hydrocortisone to PGF$_{2\alpha}$ and insulin, however, caused only partial suppression of the increase in DNA synthesis measured in the same period (40% inhibition with hydrocortisone at 40 ng ml^{-1}), even at high glucocorticoid concentrations (Fig. 1). Also the concentration of hydrocortisone required to produce half maximal inhibition of DNA synthesis was increased from 1 to 4 × 10^{-8} M in the presence of insulin (Fig. 1 insert). Addition of hydrocortisone alone or with insulin caused no stimulatory effect (Fig. 1). Preincubation of the quiescent cultures for 24 h with hydrocortisone (40 ng ml^{-1}) did not prevent the cells (95%) from synthesising DNA after addition of 10% serum.

The inhibitory effect of hydrocortisone on the stimulation of DNA synthesis produced by PGF$_{2\alpha}$ or PGF$_{2\alpha}$ and insulin was relatively specific for glucocorticoids. Thus increasing concentrations of dexamethasone as well as hydrocortisone inhibited the PGF$_{2\alpha}$-stimulation, whereas oestradiol, cortisone and testosterone were without effect (Fig. 2). Progesterone with or without insulin respectively caused only a marginal decrease or increase in the stimulatory effect of PGF$_{2\alpha}$. The concentration of dexamethasone required to cause 50% of the final reduction in DNA synthesis with PGF$_{2\alpha}$ was approximately ten times less than the corresponding hydrocortisone concentration. This correlates well with the difference in their relative glucocorticoid activities[20]. The failure of cortisone to inhibit PGF$_{2\alpha}$-stimulated DNA synthesis may be explained in part by the failure of 3T3 cells to convert cortisone into its active form, hydrocortisone[20]. Addition of hydrocortisone (400 ng ml^{-1}) to quiescent 3T6 cells plated in the absence of serum also caused a reduction in the fraction of cells synthesising DNA after a 24-h exposure to PGF$_{2\alpha}$ and insulin (Table 1). Thus the inhibitory effect of hydrocortisone was not due to the steroid interacting with other agents or hormones present in serum.

The capacity of hydrocortisone to inhibit DNA synthesis induced by PGF$_{2\alpha}$ was also reflected in its inhibition of cell multiplication. Hydrocortisone inhibited the increase in 3T3 cell numbers induced by PGF$_{2\alpha}$ or PGF$_{2\alpha}$ plus insulin after 48 h when added to quiescent cultures. Similar results were obtained when hydrocortisone was added to rapidly proliferating cells (not shown). Oestradiol at the same concentrations had no effect (Fig. 2*a*). That PGF$_{2\alpha}$ was acting directly on the cells and not through any intermediary metabolites and that hydrocortisone directly antagonised its action was suggested as follows. (1) Indomethacin (5 µM), which blocks the synthesis

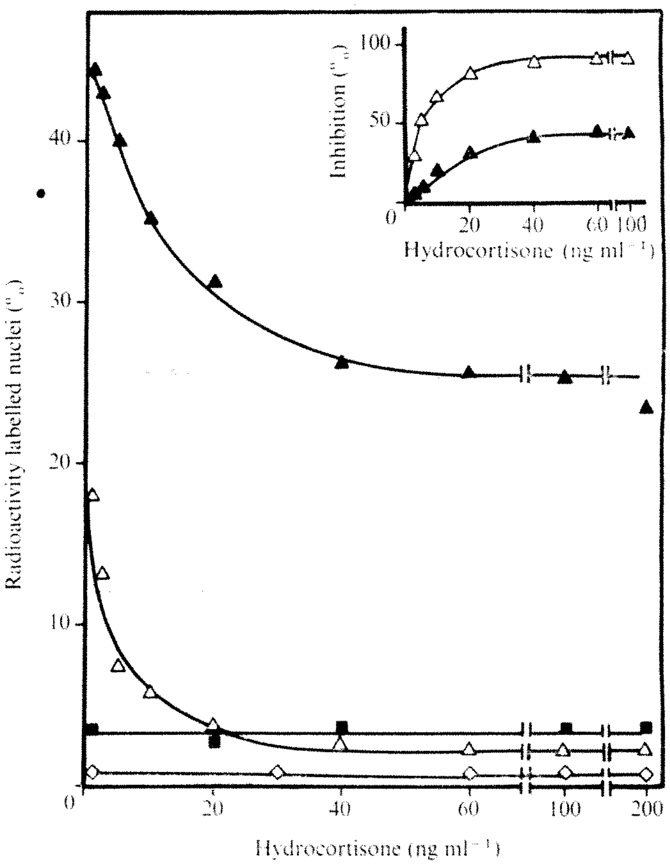

Fig. 1 Effect of various concentrations of hydrocortisone on the stimulation of DNA synthesis by PGF$_{2\alpha}$ and insulin. Additions to the cultures were as follows: PGF$_{2\alpha}$, 500 ng ml^{-1} (△); insulin, 50 ng ml^{-1} (■); PGF$_{2\alpha}$ and insulin (▲) and a control of no additions (◇) for increasing concentrations of hydrocortisone. The insert shows the percentage inhibition of the stimulation of DNA synthesis produced by hydrocortisone with either PGF$_{2\alpha}$ (△) or PGF$_{2\alpha}$ and insulin (▲). Swiss mouse 3T3 fibroblasts[31] were maintained in Dulbecco's modified Eagle's medium (DEM) containing penicillin (100 U ml^{-1}), streptomycin (100 µg ml^{-1}) and 10% foetal calf serum in 90-mm Nunc Petri dishes as described before[19]. For the determination of radioactively labelled cell nuclei, the cells were plated at 10^5 per 33-mm dish in 2 ml of DEM supplemented with 0.03 µM biotin, 0.05 µM vitamin B$_{12}$, 140 µM aspartic acid, 0.3 mM glutamic acid, 50 µM glutathione, 60 µM hypoxanthine and 5% foetal calf serum. The presence of the additional low molecular weight nutrients increased the fraction of cells synthesising DNA in the presence of PGE$_{2\alpha}$ alone with insulin when compared with those values reported[19] without supplementation[32] (M. K. O., D. C., P. S. R. and L. J. de A. in preparation). Methyl-^{3}H-thymidine was added to the medium at 3 µCi ml^{-1}, 1 µM from 0 until 26 h after the additions and the cells were continuously exposed to the extra additions until the cultures were processed for autoradiography as described before[18,19].

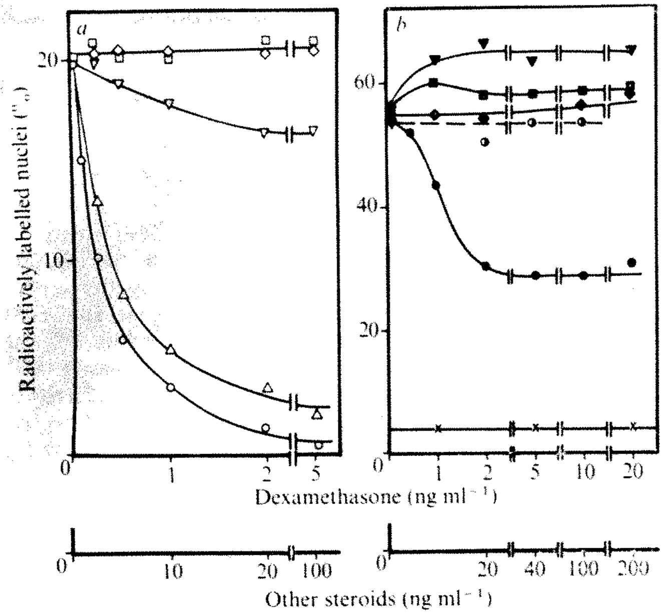

Fig. 2 Effect of various steroids on the stimulation of DNA synthesis induced by $PGF_{2\alpha}$ and insulin. *a*, Increasing concentrations of dexamethasone (○) (upper scale), hydrocortisone (△), cortisone (□), testosterone (◇) and progesterone (▽) (all on lower scale) were added to 3T3 cultures containing $PGF_{2\alpha}$ (500 ng ml^{-1}). *b*, Increasing concentrations of dexamethasone (●) (upper scale), cortisone (■), testesterone (◆), progesterone (▼), and oestradiol (◑) (all on lower scale) were added, together with $PGF_{2\alpha}$ and insulin (50 ng ml^{-1}). A control with insulin and dexamethasone alone is shown (×). The cells were radioactively labelled for autoradiography from 0 until 26 h after additions as described in Fig. 1.

Table 2 Effect of hydrocortisone on the stimulation of DNA synthesis by prostaglandin $F_{2\alpha}$ and insulin in 3T6 cells

Additions	DNA synthesis (% labelled nuclei)
None	1.3
$PGF_{2\alpha}$	14.0
Insulin	7.0
$PGF_{2\alpha}$ + insulin	31.0
$PGF_{2\alpha}$ + hydrocortisone	2.8
$PGF_{2\alpha}$ + insulin + hydrocortisone	12.4
Serum	100.0

Stock plates of Swiss mouse 3T6 cells[31] were grown in DEM with 10% foetal calf serum in 90-cm Petri dishes until the cultures were nearly confluent. The medium was removed, the cell monolayers were rinsed with 5 ml phosphate-buffered saline (0.17 M NaCl; 0.005 M KCl; 0.01 M sodium phosphate, *p*H 7.0), and the cultures were digested with 2 ml PBSA containing trypsin 100 μg ml^{-1}) for 1 min at 37 °C. The trypsin solution was then removed, and the cells were resuspended in 2.5 ml of DEM containing 0.1% serum and soybean trypsin inhibitor (200 μg ml^{-1}, Sigma, T 9003) and collected by centrifugation[32]. Cells were resuspended in 5 ml DEM and 0.1% serum, diluted 40-fold into DEM containing the extra nutrients described in Fig. 1, and then plated in 2 ml of medium at 1.5 × 10^5 cells per 33 mm dish. Final serum concentration was 0.0025%. Additions of PGF (500 ng ml^{-1}), insulin (50 ng ml^{-1}), hydrocortisone (40 ng ml^{-1}) and serum 10% were made 4 d later as indicated. The fraction of cells synthesising DNA 24 h later was recorded as described in Fig. 1.

of prostaglandins from their precursors in canine spleen[21], failed to inhibit the $PGF_{2\alpha}$-stimulated increase in DNA synthesis with or without insulin, while polyphloretin phosphate and SC 19220, inhibitors of the action of prostaglandins[22], also inhibited the mitogenic effect of $PGF_{2\alpha}$ in 3T3 cells (Table 1). (2) Stable analogues of the intermediary endoperoxides G_2 and H_2, which have similar physiological effects to $PGF_{2\alpha}$ in bronchoconstriction in animals[23] failed to stimulate cell proliferation with or without insulin present. (3) Possible antimitogenic prostaglandins which might have been synthesised or released after addition of hydrocortisone, such as PGE_1 and PGE_2 at low concentrations, failed to inhibit the mitogenic effect of $PGF_{2\alpha}$ or $PGF_{2\alpha}$ plus insulin in 3T3 cells (unpublished results).

The mechanism of action of the glucocorticoid-induced inhibition of DNA synthesis and its partial reversal by insulin is unknown, although glucocorticoid-induced reductions in glucose[24] rubidium and phosphate[25] uptakes have been reported in different cells and these transport systems are stimulated by $PGF_{2\alpha}$ or insulin in mouse fibroblasts[26]. Transport alterations probably do not mediate directly the inhibitory effect of glucocorticoids, since their reduction of the uptake of rubidium ions and phosphate is dependent on protein synthesis in rat thymocytes[25]. Furthermore, the growth of mouse L929 cells in medium containing high concentrations of fructose instead of glucose where the sugar enters the cell by passive diffusion rather than by carrier mediated transports[24] can also be inhibited by glucocorticoids. It is surprising that hydrocortisone, a predominantly catabolic hormone[12] can increase cell proliferation with FGF in fibroblastic cells[17,18], or with prolactin in mammary epithelial cultures[10]. Hydrocortisone, however, may

Table 1 Effect of inhibitors and analogues of $PGF_{2\alpha}$ on DNA synthesis and cell division

Additions	DNA synthesis (% ^{3}H-labelled nuclei)	Cell no. (× 10^{-5})
None (control)	0.2	6.03 ± 0.22
$PGF_{2\alpha}$	18.0	7.57 ± 0.03
$PGF_{2\alpha}$ + hydrocortisone	1.7	6.28 ± 0.22
$PGF_{2\alpha}$ + insulin	41.0	14.81 ± 1.1
$PGF_{2\alpha}$ + insulin + hydrocortisone	23.0	9.19 ± 0.4
$PGF_{2\alpha}$ + PPP	1.2	6.02 ± 0.0
$PGF_{2\alpha}$ + insulin + PPP	22.0	7.87 ± 0.6
$PGF_{2\alpha}$ + SC-19220	5.2	6.89 ± 0.6
$PGF_{2\alpha}$ + indomethacin (2.5 μM)	19.0	—
$PGF_{2\alpha}$ + indomethacin (5.0 μM)	17.7	8.85 ± 1.0
$PGF_{2\alpha}$ + insulin + indomethacin (2.5 μM)	42.0	—
$PGF_{2\alpha}$ + insulin + indomethacin (5.0 μM)	39.0	12.8 ± 0.3
Insulin	3.9	6.5 ± 0.6
U-44069	0.2	5.6 ± 0.2
U-46619	0.2	6.3 ± 0.1
Serum	97	24.50 ± 1.2
Serum + hydrocortisone	97	23.9 ± 0.2

3T3 cultures were plated at 10^5 cells per 33-mm Petri dish for the determination of DNA synthesis or 1.5 × 10^5 cells in 5 ml of medium per 5-cm Petri dish for determination of cell numbers as described in Fig. 1. After 4 d, when the cultures became stationary, $PGF_{2\alpha}$ (500 ng ml^{-1}); hydrocortisone (40 ng ml^{-1}), insulin (50 ng ml^{-1}); polyphloretin phosphate[22] (PPP, 5 μg ml^{-1}); 1-acetyl-2-(8-chloro-10,11-dihydrodibenz(*b,f*)(1,4)oxazepine-10-carbonyl)hydrazine[22] (SC 19220) (10 μg ml^{-1}); indomethacin 2.5 or 5 μM; (15S)-hydroxy-9α,11α(epoxymethano) prosta-5z,13-dienoic acid[23] (U-44069, 500 ng ml^{-1}); (15S)-hydroxy-11α,9α(epoxymethano) prosta-5z, 13-dienoic acid[23] (U-46619, 500 ng ml^{-1}), and 10% foetal calf serum were added as indicated. The fraction of cells synthesising DNA by 26 h or the numbers of cells ± s.e. observed by 48 h after additions is shown. In controls the amount (μg) of DNA isolated[33] per 10^6 cells 40 h after the addition of: nothing; $PGF_{2\alpha}$; $PGF_{2\alpha}$ + hydrocortisone; $PGF_{2\alpha}$ + insulin; $PGF_{2\alpha}$ + insulin + hydrocortisone; $PGF_{2\alpha}$ + insulin + indomethacin; serum was 11.6 ± 0.4; 15.0 ± 0.4; 12.2 ± 1.1; 19.3 ± 0.3; 14.1 ± 1.5; 19.2 ± 0.4 and 18.7 ± 2.1 respectively.

increase the efficiency of pituitary polypeptide interaction with the membrane in a manner similar to the way in which it specifically enhances lipolytic action of pituitary ACTH in fat cells[27].

In conclusion, both positive and negative effects of corticosteroids on the growth of cultured mouse fibroblasts have been reported. The same concentrations of hydrocortisone can either increase the rate of DNA synthesis induced by FGF (refs 17, 18) or decrease the rate induced by PGF$_{2\alpha}$ in the same Swiss mouse 3T3 cell cultures. Thus the cell growth regulatory properties of the glucocorticoids can be determined by the identity of the co-inducing growth controlling agents: this may in part explain their paradoxical effects in tissue culture. The exact physiological relevance of these observations is unknown. *In vivo* corticosteroids are potent anti-inflammatory agents[28], while prostaglandins may mediate some of the effects of the inflammatory process[21]. Hydrocortisone can inhibit both PGF$_{2\alpha}$-stimulated cell growth and collagen production in fibroblasts[28,29], so corticosteroid anti-inflammatory activity against prostaglandins may involve direct competition for control of various cellular processes including cell multiplication, and may not be solely due to inhibition of the production of prostaglandins[30].

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Altered cyclic AMP-binding and db cyclic AMP-unresponsiveness *in vivo*

CYCLIC AMP has been implicated in the regulation of cell growth. However, the molecular mechanism of cyclic AMP action, especially that involving the control of tumour growth *in vivo* is not clear. Our previous studies on two cell populations of Walker 256 mammary carcinoma (W256), one regressing (responsive), the other growing (unresponsive) under dibutyryl (db) cyclic AMP treatment, showed a correlation between altered cyclic AMP-binding of the tumour cytosol and db cyclic AMP-unresponsiveness[1]. Deficient cyclic AMP-binding in other malignant growth has also been reported[2–4]. It is suggested that the defective cyclic AMP-binding proteins may be the possible cause for

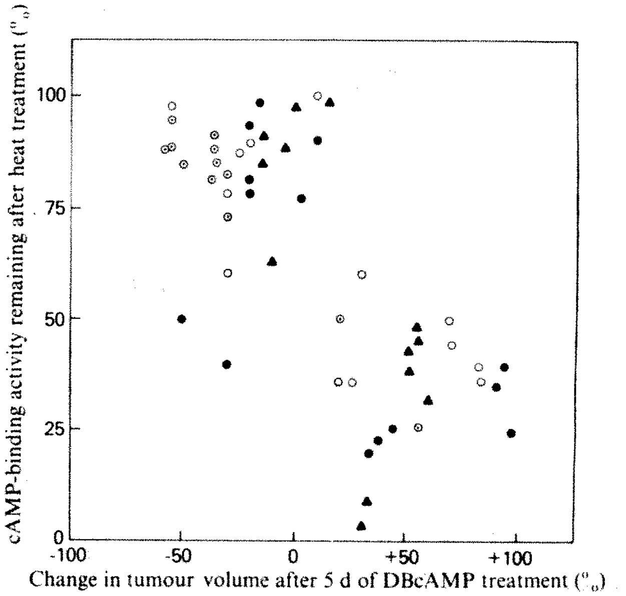

Fig. 1 Correlation between heat stability of cyclic AMP-binding and db cyclic AMP-induced tumour regression. ⊙, MTW9; ●, NMU; ○, R3230AC; ▲, hepatoma 5123. Growth of tumours was measured daily and expressed as the percentage change in volume[5]. Rats bearing 2–3-g tumours were injected[5] with db cyclic AMP for 5 d and killed 5 d later. Tumours were homogenised with 5 volumes of Tris-HCl buffer, 10 mM (*p*H 7.5). Protein concentrations of the cytosols (105,000*g*, 1 h) were adjusted to 2 mg ml^{-1} with the buffer. Aliquots of the cytosols were placed in glass tubes and heated (in duplicate) in a 50 °C water bath for 15 min stirring gently with a glass rod, then cooled to 0 °C. The unheated and heated cytosols were assayed for cyclic AMP-binding at 0 °C for 90 min by Gilman's competition protein binding assay[6] as previously described[1]. High affinity binding was determined at 10^{-8}M cyclic AMP (Y.S.C.-C. *et al.*, in preparation). Tumour cytosols were also heated as above after pre-incubation of cytosols with non-radioactive cyclic AMP (10^{-7} M) at 0 °C for 90 min. Both heated and unheated cytosols which were presaturated with cold cyclic AMP, were then treated with Dextran coated charcoal (DCC) to remove unbound cyclic AMP (Y.S.C.-C. and T.C., in preparation). One ml (2 mg protein) of each cytosol was added to a 400*g* DCC pellet derived from 4.0 ml of the DCC suspension (0.25% Norit A and 0.0025% Dextran, Grade C, in 0.01 M Tris-HCl, *p*H 8.0); the mixtures were incubated in a 23 °C water bath for 15 min with intermittent shaking every 5 min, then cooled to 0 °C, and centrifuged. The supernatants were assayed for cyclic AMP-binding at 2×10^{-8} M cyclic AMP by the modified method of Do Khac *et al.*[7] to enhance the cyclic AMP exchange. The reaction was carried out at 23 °C for 3 h. The specific binding activity[8] was expressed as the percent activity of the unheated control. Each symbol represents the average of duplicate determinations of a single tumour. Tumours frozen at −70 °C for up to 2 weeks gave results similar to those on unfrozen material. The heat stability test was also done with tumour biopsy specimens before db cyclic AMP treatment. The same correlation between the stability of the binding proteins and growth arrest was obtained. Protein concentrations were determined by the method of Lowry *et al.*[9]

the loss of growth control by cyclic AMP. To determine the relationship between the qualitative difference of cyclic AMP-binding proteins and db cyclic AMP-responsiveness *in vivo*, we have used various experimental tumour models, such as, transplantable mammary carcinoma (MTW9) in Wistar-Furth inbred female rats, a hormone-dependent tumour; primary, *N*-nitrosomethylurea-induced mammary carcinoma (NMU) and transplantable mammary adenocarcinoma (R3230AC) in Fischer female rats, hormone-responsive tumours; and hepatoma 5123 in Buffalo/N female rats.

Figure 1 shows a relationship between heat stability of cyclic AMP-binding proteins in tumour cytosol and db cyclic AMP-responsiveness *in vivo* with 4 types of rat tumours. The high-affinity binding activity (Y.S.C.-C., *et al.*, in preparation) of the cytosols from unresponsive tumours (growing under db cyclic AMP treatment) was reduced to 50% or less when the cytosol was pre-incubated at 50 °C for 15 min; under the same treatment, more than 75% of the binding activity of the cytosols from responsive tumours (regressing under db cyclic AMP treatment) was retained. Mixing of cytosols from both responsive and unresponsive tumors gave additivity in the heat stability, suggesting that the heat lability of unresponsive tumour binding was not due to enzymatic degradation of the binding proteins. Presaturation of free binding sites with unlabelled cyclic AMP did not alter the stability of the binding proteins; this suggests that the heat stability of the binding activity of the responsive tumours was not due to high cyclic AMP concentrations in the tumour cytosols which might protect against the inactivation of the binding protein. Moreover, 8 mM *β*-mercaptoethanol decreased the heat lability of the binding in the unresponsive tumours but had little or no effect on the stability of the binding in the responsive tumours. Thus, there seems to be a difference in the structure or conformation of the binding proteins between the responsive and the unresponsive tumours. For 44 of 52 tumours there was a striking parallel between stable cytoplasmic cyclic AMP-binding proteins and db cyclic AMP-induced tumour regression *in vivo* (Fig. 1). A correlation between heat stability of cyclic AMP-binding proteins and db cyclic AMP-responsiveness has also been found in cultured neuroblastoma cells by Simantov and Sachs[10] who reported that cells resistant to the cytotoxic effect of db cyclic AMP had cyclic AMP-binding proteins more sensitive to temperature than non-resistant cells.

The results in Fig. 2 show that the thermal lability of cyclic AMP-binding is associated with a reduced affinity of the binding proteins for cyclic AMP. The specific binding curves of db cyclic AMP-responsive and -unresponsive R3230AC tumours showed that the binding proteins in unresponsive tumour had a reduced apparent affinity for cyclic AMP, but the maximum binding sites in both tumours were similar. Scatchard analysis[11] of the binding curves gave K_d of 2.0×10^{-8} M and 1.2×10^{-7} M, for responsive and unresponsive tumours, respectively (Fig. 2, left, inset). It has been shown that cyclic AMP-binding proteins are a regulatory subunit of many mammalian protein kinases and the binding of cyclic AMP to the regulatory subunit stimulates the kinase activity[13-19]. Fig. 2 shows that cyclic AMP stimulation of protein kinase is apparently reduced in unresponsive tumour, whereas the K_m of the enzyme for ATP in the presence or absence of cyclic AMP is similar in responsive and unresponsive tumours (Fig. 2, right). These data are consistent with the difference in binding affinity for cyclic AMP observed between responsive and unresponsive tumours (Fig. 2, left).

The depression of cytosol binding activity of responsive W256 after db cyclic AMP treatment could be the result of an increase in endogenous binding of unlabelled cyclic AMP which decreases the exogenous ^{3}H-cyclic AMP-binding[2]. Alternatively, the decrease in the binding by the cytosol may reflect the change in intracellular compartmentalisation of binding protein and an actual loss of binding protein from the cytosol after db cyclic AMP treatment. These possibilities were examined by measuring cyclic AMP-binding and protein kinase actvites in cytosols and nuclei from db cyclic AMP-treated and control tumours using sucrose gradient centrifugation (Fig. 3). Cytosol from the db cyclic AMP-responsive R3230AC control (*a*) exhibited two major peaks of cyclic AMP-binding activity, sedimenting at 4.3S and 5.6S, respectively. The cytosol also contained cyclic AMP-independent and -dependent forms of protein kinase, sedimenting at 3.3S and 5.6S, respectively. The latter sedimentation constant is identical to that of the heavier cyclic AMP-binding peak. Cytosol from the db cyclic AMP-unresponsive control tumour (*e*) showed a different sedimentation pattern of cyclic AMP-binding and protein kinase activities: the binding component sedimenting at 4.3S and the cyclic AMP-independent protein kinase sedimenting at 3.3S decreased while the higher molecular weight species of these proteins which were not apparent in the responsive tumour cytosol increased

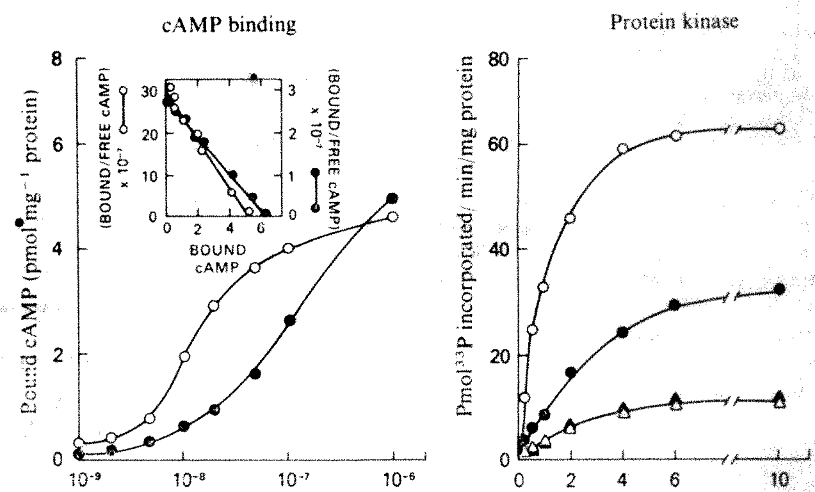

Fig. 2 Cyclic AMP-binding and protein kinase activities of db cyclic AMP-responsive (open symbols) and -unresponsive (closed symbols) R3230AC tumours. ○, ●, Cyclic AMP binding or protein kinase activity in the presence of cyclic AMP; △, ▲, Protein kinase activity in the absence of cyclic AMP. Db cyclic AMP-responsive and -unresponsive tumours were obtained from standard R3230AC transplants following a method previously described[12]. The preparation of tumour cytosol was the same as in legend to Fig. 1. Cyclic AMP binding was assayed at various concentrations of cyclic AMP as previously described[1]. Protein kinase activity was determined by the measurement of ^{33}P incorporation from γ-labelled ATP into histone. The reaction mixture (0.25 ml) contained: 100 mM potassium phosphate buffer (*p*H 7.5); 20 mM magnesium acetate; 1 mM theophylline; 0.6 mg calf thymus histone; 3×10^{-7} M (γ-^{33}P) ATP, 19 Ci mmol^{-1} (ICN Pharmaceuticals, Inc., Pikesville, Md.) with various concentrations of non-radioactive ATP; and cytosol (70 μg protein). Incubations were carried out at 30 °C for 5 min ± 1 μM cyclic AMP and the reactions were stopped by the addition of 0.5 ml of ice-cold 20% TCA. After standing for 30 min in an ice bath, the samples were filtered on to Millipore filters (0.45 μm). The filters were then washed 6 times with 5 ml of 5% TCA, dried and counted by liquid scintillation. Values represent one of the 2 similar experiments and 5 pooled tumours were used in each experiment.

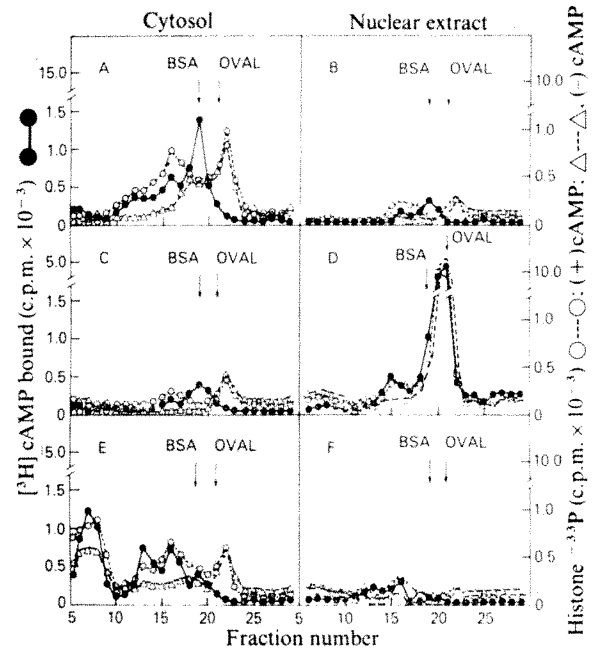

Fig. 3 Sucrose density gradient centrifugation of cytosols and nuclear extracts of db cyclic AMP-responsive and -unresponsive R3230AC mammary tumours before and after db cyclic AMP treatment. *a*, *b*, db cyclic AMP-responsive control; *c*, *d*, db cyclic AMP-responsive treated (db cyclic AMP treatment[5] for 5 days); *e*, *f*, db cyclic AMP-unresponsive control (db cyclic AMP-unresponsive-treated was similar to *e* and *f*). See description of Fig. 2 for db cyclic AMP-responsive and -unresponsive tumours. Tumours were homogenised in a Teflon–glass homogeniser with 2 volumes of 10 mM Tris-HCl buffer (*p*H 7.5). The supernatants (105,000*g* for 1 h) were used as cytosols. The pellets from 105,000*g* were rehomogenised in an original volume of 20 mM Tris-HCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, *p*H 7.5 (buffer A). These homogenates were passed through 3 layers of gauze and centrifuged at 770*g* for 10 min. The pellets were resuspended and homogenised in the same volume of buffer A, then centrifuged at 770*g* for 10 min. The nuclear pellets thus obtained were homogenised in one volume of 0.4 M KCl in 10mM Tris-HCl buffer (*p*H 7.5) and extracted at 0 °C for 90 min. The suspensions were centrifuged at 10,000*g* for 10 min and the clear supernatants used as nuclear extracts. Samples of cytosols and nuclear extracts containing 1.0 mg of protein were incubated at 23 °C for 3 h, with 10^{-6} M [³H]-cyclic AMP, 10 mM theophylline and 50 mM potassium phosphate buffer (*p*H 7.0) then layered on 5–20% sucrose gradient in 10 mM Tris-HCl buffer (*p*H 7.0) (nuclear gradient included 0.4 M KCl). Centrifugation was at 2 °C for 17 h at 45,000 r.p.m. in a Beckman SW 50.1 rotor. Thirty fractions were collected and assayed for cyclic AMP-binding (protein-bound ³H content) and for protein kinase activity. For the determination of cyclic AMP-binding, aliquots of each fraction were filtered onto Millipore filters (0.45 μm), washed and counted by liquid scintillation. Protein kinase activity was determined as in the legend to Fig. 2 at 0.5 mM ATP concentration. Sedimentation coefficients were determined by the method of Martin and Ames[20] using BSA (Bovine Serum Albumin, $S_{20,w} = 4.5$S) and OVAL (Ovalbumin, $S_{20,w} = 3.6$S) as internal standards. The values represent one of the 3 similar experiments.

markedly. The sedimentation profiles of cyclic AMP-binding and protein kinase of control nuclei from both responsive and unresponsive tumours (*b* and *f*) showed small broad peaks. In contrast, the nuclear extract from the db cyclic AMP-treated-responsive tumour (*d*) gave a sharp sedimentation profile composed mainly of a 3.6S species containing both cyclic AMP-binding and cyclic AMP-independent protein kinase activities. This increase in nuclear binding and kinase activities was accompanied by a decrease of these protein species in the cytoplasm (*c*).

The nuclear accumulation of cyclic AMP-binding and protein kinase components was not found in the db cyclic AMP-unresponsive tumour following the db cyclic AMP treatment; the sedimentation profiles of these macromolecular protein species in both the cytosol and nuclear extracts were the same as those in the untreated control tumour. The differences observed between db cyclic AMP-treated-responsive and -unresponsive tumour nuclei with respect to cyclic AMP-binding and protein kinase activities was not due to the presence of a modulator of the binding and kinase in the extracts since mixing of db cyclic AMP-treated-responsive and -unresponsive tumour nuclear extracts exhibited additivity of the binding and kinase activities. A similar correlation between the sedimentation characteristics of cyclic AMP-binding proteins and protein kinase and responsiveness to db cyclic AMP treatment was found with db cyclic AMP-responsive and -unresponsive 5123 hepatomas (data not shown).

Our studies have revealed a striking parallel between heat stability of cyclic AMP-binding and arrest of growth due to exogenous db cyclic AMP. Moreover, a close correlation was found between the heat stability of cyclic AMP-binding and affinity of the binding proteins for cyclic AMP: the binding proteins which are more stable to temperature possess a higher affinity for cyclic AMP than heat labile binding proteins. These qualitative differences of cyclic AMP-binding proteins between db cyclic AMP-responsive and -unresponsive tumours also reflect their capability for nuclear accumulation: only those binding proteins which are heat stable to temperature and exhibit a higher affinity for cyclic AMP accumulate into nuclei during db cyclic AMP treatment. These findings indicate that 'unresponsiveness' to db cyclic AMP treatment is closely associated with qualitative defects in cyclic AMP binding proteins. Thus the heat stability test of cytosol cyclic AMP-binding proteins could be used as a simple diagnostic method to assess the efficacy of cyclic AMP treatment of tumours.

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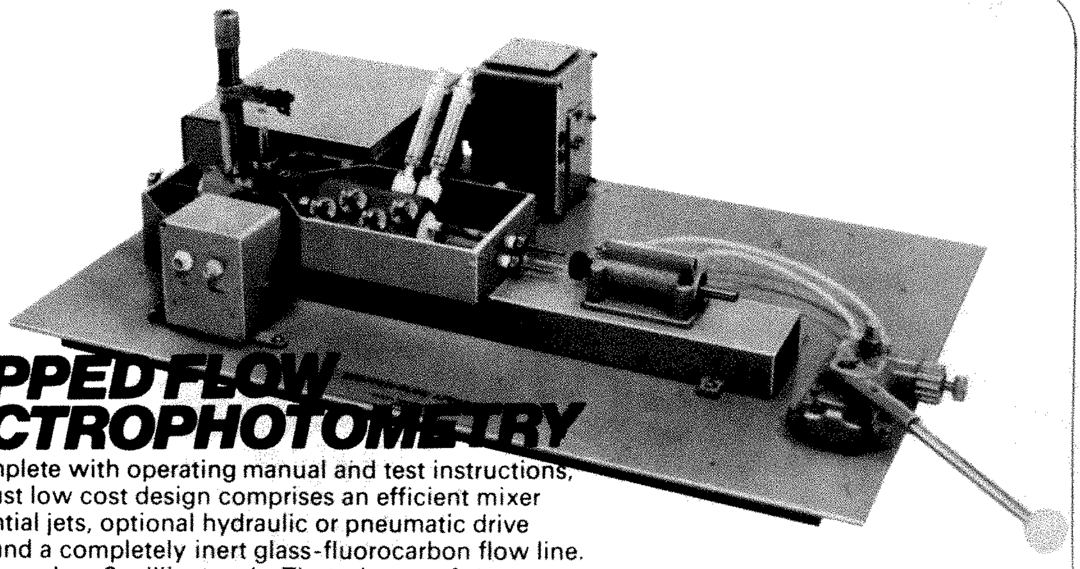
Prevention of senescence in the ascomycete *Podospora anserina* by the antibiotic tiamulin

THE syndrome of senescence or aging is common to all living material. In many fungi the peripheral hyphae become senescent after prolonged vegetative growth, that is, their growth rate decreases and they eventually die. In some fungi, senescence is caused by transmissible elements, of temporarily if their metabolism is drastically reduced[1,3], but



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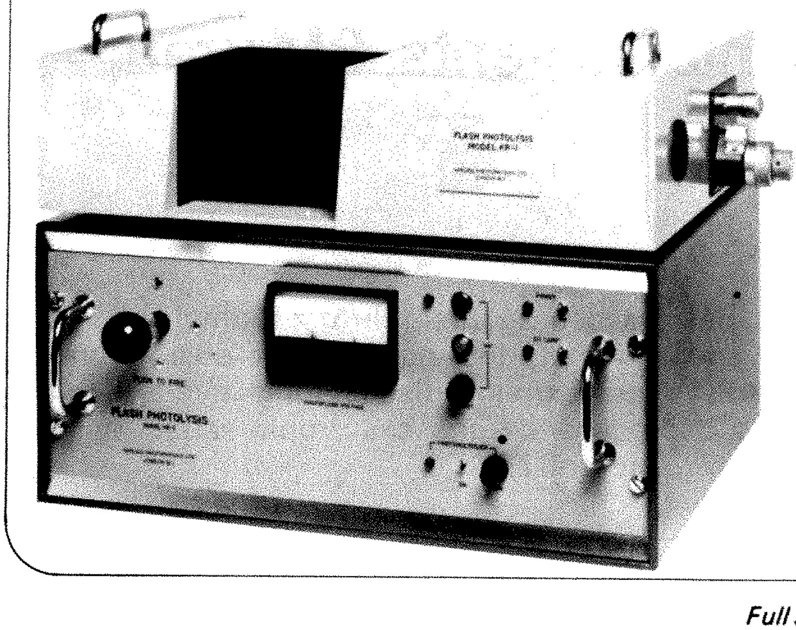

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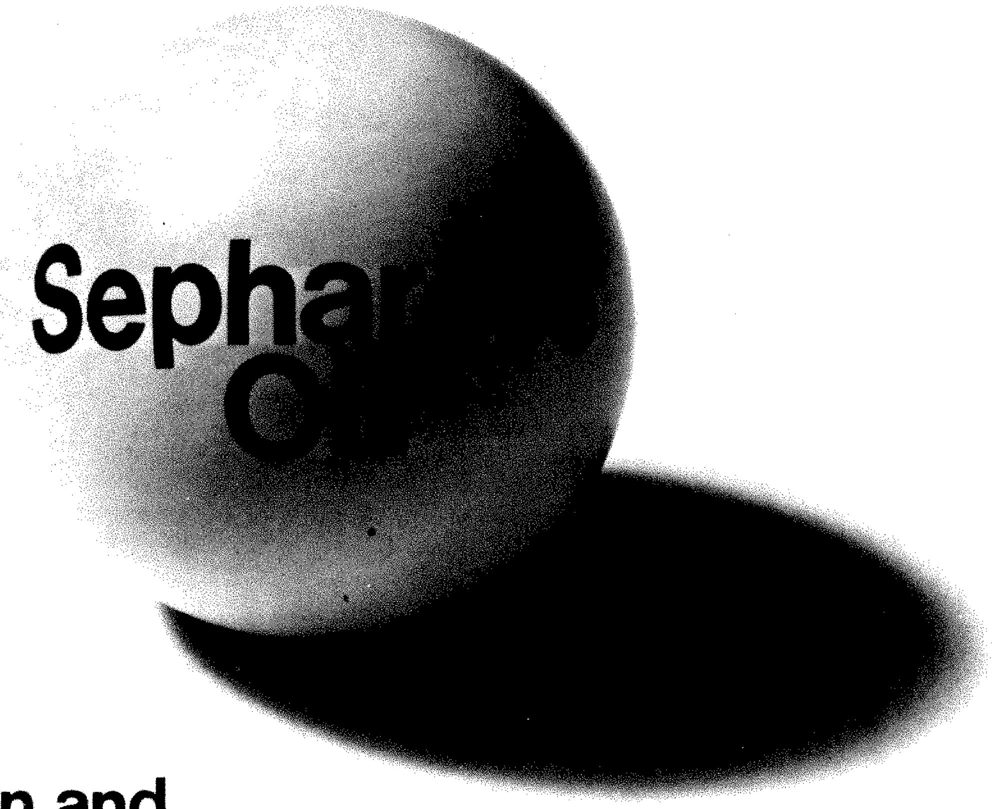

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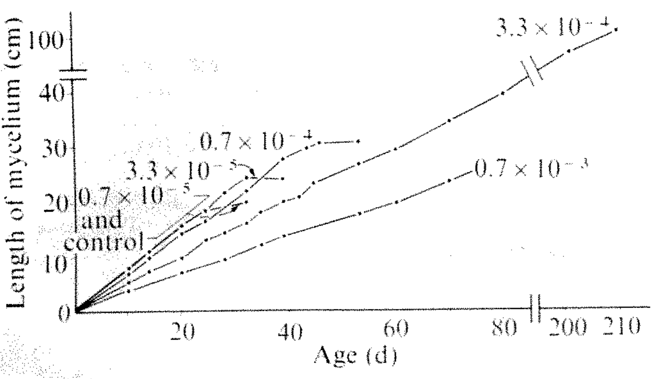
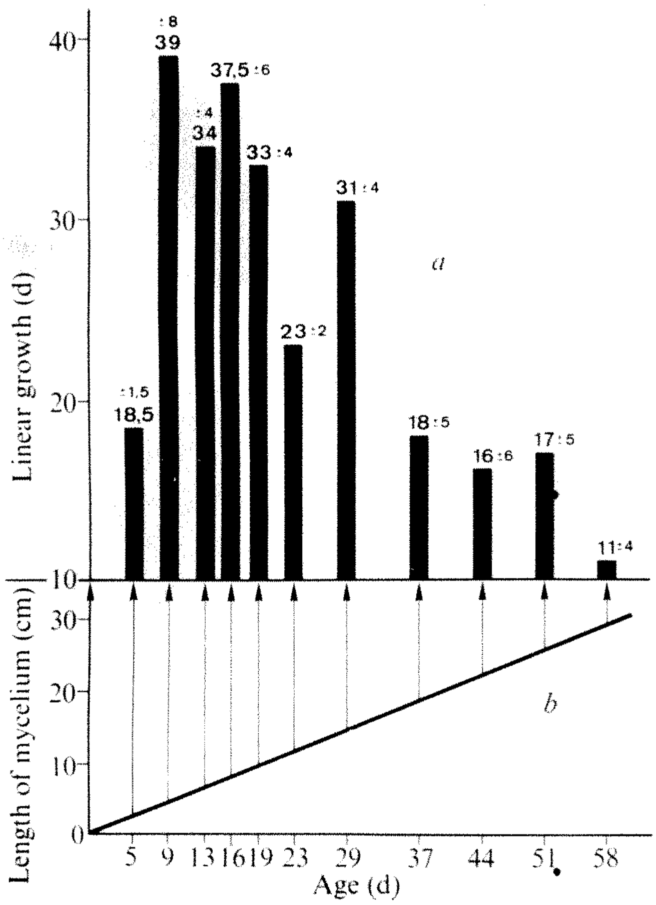


Fig. 1 Effect of the antibiotic tiamulin on the growth of *P. anserina.* Mycelia of the wild strain s$_1$ were grown at 25 °C in race tubes (length 50 cm) on cornmeal agar containing tiamulin as indicated. The growth curves were calculated from at least five parallel runs which were inoculated with mycelia 'freshly' regenerated by sexual propagation.

anastomosis. The onset of senescence is influenced by environmental as well as genetical factors[1–6]. In *Podospora anserina,* where senescence has been very thoroughly analysed, it was shown that senescent mycelia may recover temporarily if their metabolism is drastically reduced[1,5], the mycelia soon become senescent when grown in normal conditions. The nature of the senescent agent in *Podospora* is obscure. All efforts to demonstrate viruses or virus-like particles in aging hyphae of *Podospora* have failed (R. Bozarth, personal communication). Our endeavours to determine the nature and action of the senescent agent were encouraged by previous findings that the onset of senescence can be completely prevented by a synergistic action of specific genes[6]. In this paper we show that an antibiotic, tiamulin, can mimic the effect of these genes.

Fig. 2 Correlation between the length of time the mycelium has grown on tiamulin and the onset of senescence. *a*, Time of linear growth of samples taken at various intervals (arrows) and transferred to medium devoid of tiamulin. *b*, Growth curve of the mycelium kept on tiamulin (3.3 × 10^{-4} M). For experimental conditions see legend to Fig. 1.



Informative experiments showed that when some tetracyclines were incorporated into the agar medium they induced a 'stopper phenotype'[3], thus prolonging the life of the mycelium. In contrast, cholesterol reduced the life span by 30–50%. It is known that tetracyclines inhibit and cholesterol promotes the growth of mycoplasmas[7,8]. It is thus possible that these organisms could be involved in the senescence syndrome in *Podospora.*

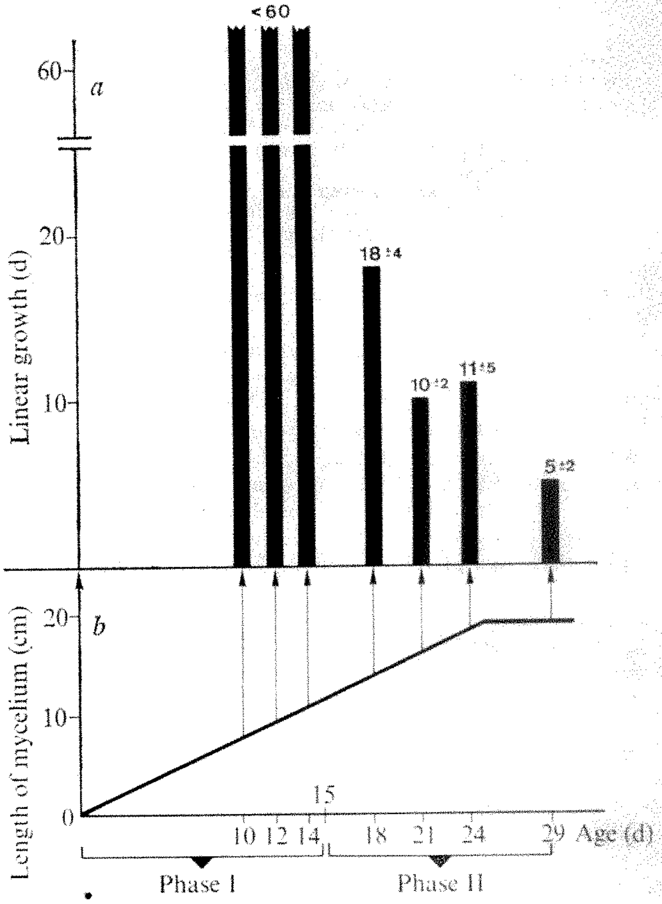


Fig. 3 Correlation between the age of mycelium and its sensitivity to tiamulin treatment. *a*, Time of linear growth of samples taken at various intervals (arrows) and transferred to medium containing 3.3 × 10^{-4} M tiamulin. *b*, Growth curve of mycelium grown on medium devoid of tiamulin. For experimental conditions see legend to Fig. 1.

This idea is substantiated by the results of infection experiments. When senescent and juvenile mycelia were mixed in a Waring blender and incubated at 25 °C for 24 h, a 100% infection was obtained only in media with a high osmotic value (20% sucrose); at low osmotic values (1–5% sucrose) few or no juvenile hyphae were infected. This osmotic sensitivity of the senescent agent is well in accordance with the properties of mycoplasma-like organisms (prokaryotic cells devoid of a cell wall).

In order to confirm this assumption we have looked at the effect of a new antibiotic, tiamulin, on the hyphal growth of *Podospora.* This antibiotic is supposed to be specific for mycoplasms (personal communication Biochemie Kundl, Österreich). As seen from Fig. 1, tiamulin can prevent senescence. The most effective concentration is around 10^{-4} M. Lower concentrations only postpone senescence whereas higher concentrations prevent senescence but have a toxic effect and reduce the linear growth rate. In order to obtain more information on the action of tiamulin we have performed two series of experiments

(Figs 2 and 3). As can be seen from the data in Fig. 2, the time at which the onset of senescence occurs is influenced by the period for which the mycelium is first grown on tiamulin medium. There is considerable delay up till 30 d after which the situation is reversed and the onset of senescence soon occurs. Possibly the mycelium becomes dependent on tiamulin at this time. It is evident that tiamulin does not cure senescence but can prevent it when present in the medium.

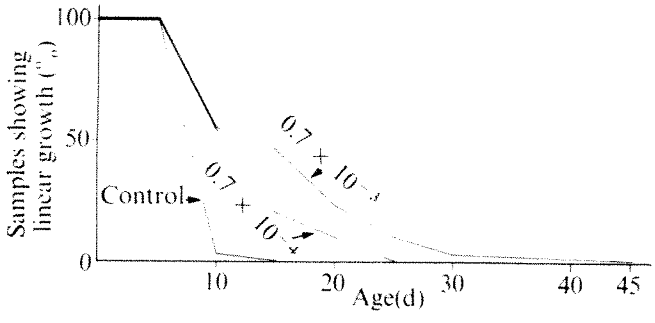


Fig. 4 Effect of various concentrations of tiamulin on senescent mycelium. Senescent mycelia grown in corn steep liquor in Fernbach flasks were homogenised in a Waring blender (60 s) in a solution of 20% sucrose containing tiamulin as indicated and incubated at 25 °C for 24 h. The life span of at least 30 samples at each concentration was followed after plating small inocula on corn meal agar. The curves are mean values showing the relative survival rates.

Figure 3 shows that the life span of *Podospora* can be roughly divided into two phases with respect to the life-prolonging effect of tiamulin. If given in phase I (0–15 d) senescence can be prevented. If given about 10 d before the onset of senescence (phase II, 15 d and later) there is no longer an effect; the mycelium continues to grow on the antibiotic for the rest of its normal life span.

These observations confirm earlier findings[1] that even before these symptoms of senescence become obvious, the senescent agent is present in the mycelium. These data allow us to conclude that tiamulin can only prevent the activity of the senescent agent up to a critical time (perhaps a particular stage of development of the agent or its concentration in the cell). After this time the agent spreads within the mycelium and after an appropriate 'incubation time' causes the senescent phenotype.

To show that the ineffectiveness of tiamulin in phase II is not a result of its reduced uptake by the aerial hyphae, a 'therapeutic' experiment was performed in liquid culture. From the data summarised in Fig. 4 it can be seen, that tiamulin is still effective to a certain extent on senescent mycelium and that this effect increases with the concentration of the antibiotic. Two interpretations are possible. First, tiamulin prevents the infection of the few juvenile hyphae still present in the senescent mycelium and allows them to grow despite the presence of neighbouring senescent hyphae. Second, tiamulin may cure infected but not severely damaged hyphae and give them a new chance to grow.

Our experiments have shown that tiamulin is effective in preventing the occurrence of senescence in *Podospora* in specific conditions. As yet we do not know whether tiamulin acts prophylactically or therapeutically, but the possibility that it is acting by killing mycoplasmas must be investigated, particularly since mycoplasma-like organisms have recently been found in a fungus[9]. The feasibility of using tiamulin and other derivatives of pleuromulin for preservation of strains and prolongation of fermentation time in the fermentation industry is under examination.

We wish to thank T. Itoh (Obihiro/Hokkaido, Japan) who contributed to the informative experiments with tetracycline and cholesterol during his stay in our laboratory as a fellow of the Heinrich-Hertz-Stiftung (Düsseldorf). Tiamulin (2-diethyl-aminoethyl)-thioacetoxy-mutilin-hydrogenfumerat) was kindly donated by Biochemie G.m.b.H. Kundl, Österreich. We thank the technical staff of our laboratory for their assistance, especially Frau U. Rojek and Frau H. Hüsemann.

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Solubilised viral proteins produce fatal hepatitis in mice

PATHOGENICITY of viruses is commonly related to their ability to multiply within the host's cells. Although, in certain conditions, the inoculation of large doses of uninfectious virus can produce a fatal disease[1], the toxicity of viral components as such for laboratory animals has never been demonstrated. We have shown that although frog virus 3 (FV 3) is unable to multiply in mice, it produces an acute degenerative hepatitis leading to the death of the animals in less than 24 h (refs 2, 3). We report here that solubilised viral proteins also produce this hepatotoxic effect.

FV 3 structural proteins were solubilised in two steps. First, a purified virus suspension was incubated with Nonidet P-40 (final concentration 0.5%) for 5 h at room temperature. The detergent was then removed through extensive dialysis in 50 mM Tris-HCl, *p*H 8.5. Second, LiCl (20%, w/w) was added to the preparation and the mixture was left for 6 h at 0 °C then dialysed again and centrifuged at 100,000*g* for 1 h. The supernatant fluid containing the soluble viral extract (SVE) was used in experiments[4].

Intravenous (i.v.) inoculation of 0.2 mg of SVE proteins per mouse killed the animals within 24–48 h (Table 1); the

Table 1 Lethal effect of the solubilised viral extract in mice

SVE proteins (mg kg^{-1})	Treatment of SVE	Animals killed/animals inoculated
10	None	6/6
5	None	5/6
2.5	None	5/6
1.25	None	1/6
0.62	None	0/6
20	80 °C, 30 min	0/6
20	Trypsin*	0/6
20	Tris-HCl	6/6
20	FV$_3$ antibodies†	0/6
20	Normal rabbit serum	6/6

IC strain mice (18–20 g) were inoculated i.v. with 0.5 ml containing various amounts of viral proteins diluted in 50 mM Tris-HCl, *p*H 8.5, 15 mM NaCl.

*SVE (1.5 mg ml^{-1}) was incubated for 1 h at 37 °C with or without trypsin (0.5 mg ml^{-1}). Before the inoculation, 100 000 peptidase inhibitory units of iniprol (Choay) were added per ml of hydrolysed or unhydrolysed SVE.

†FV 3 antibodies were produced by inoculating rabbits with purified virus supplemented with complete Freund's adjuvent. SVE was mixed with anti FV 3 serum (final serum dilution 1/100) or normal rabbit serum for a 1-h incubation at 37 °C.

LD_{50} was about 2 mg per kg body weight. This lethal action was related to SVE proteins. Both heating the SVE for 30 min at 80 °C and trypsin digestion destroyed its toxicity. The toxicity could also be neutralised by specific FV 3 antibodies (Table 1).

Histological lesions were found only in the liver. The whole lobule was affected with the exception of one or two layers of cells around the centrolobular vein. These lesions were studied by electron microscopy at intervals after the inoculation of one LD_{100} of SVE. As early as 3 h afterwards, the nuclei of the hepatocytes were severely damaged; they showed an irregular shape, condensation of the chromatin, assembly of the interchromatin granules in dense clusters at the centre of the nucleus, and fibrillar inclusions (Fig. 1*a*).

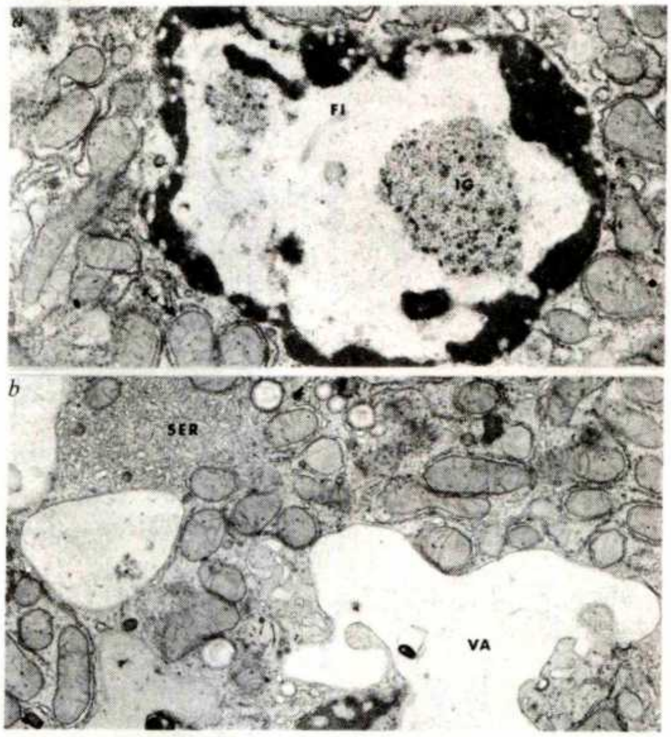

Fig. 1 Ultrastructural changes produced in hepatocytes of mice inoculated i.v. with SVE 10 mg per kg body weight. *a*, Hepatocyte nucleus observed 3 h after the inoculation. The condensation of the chromatin (CH) and the emptiness of the nucleoplasm appear clearly. The interchromatin granules (IG) are assembled into a dense cluster at the centre of the nucleus and some fibrillar inclusions (FI) are noticeable. 7,200. *b*, Part of the cytoplasm of a hepatocyte 9 h after inoculation. The cytoplasm shows numerous vacuoles (VA) as well as large areas of smooth endoplasmic reticulum (SER) (9,1120).

Cytoplasmic changes were only noticeable 9 h after the inoculation. They were characterised by alterations of both rough and smooth endoplasmic reticulum, accumulation of lipids and a final necrosis (Fig. 1*b*).

As with inoculation of virus particles, the macromolecular metabolism of the liver was markedly affected by SVE injection. Table 2 shows that both RNA and protein synthesis were depressed 3 h after inoculation, RNA synthesis being more severely affected.

Thus the inoculation of solubilised structural proteins from FV 3 produces the same morphological and biochemical alterations of the liver as the intact virions. Our results clearly demonstrate that viral proteins may be 'toxic' by themselves. Although it is unlikely that an organism would be naturally infected with an amount of virus sufficient to produce extensive lesions by this mechanism, most pathogenic viruses undergo several cycles of multiplication enabling toxic proteins to accumulate.

Table 2 Effect of SVE on RNA and protein syntheses in mouse livers

Time after inoculation (h)	c.p.m. incorporated	
	^{3}H-orotic acid	^{14}C-amino acid
0	147,680 (100)	269,520 (100)
3	81,200 (55)	180,560 (67)
6	59,040 (40)	161,680 (60)
9	51,680 (35)	134,760 (50)

Mice were inoculated i.v. with SVE at 10 mg per kg body weight. The mice received 50 μCi of ^{3}H-orotic acid (specific activity 22 Ci mmol^{-1}) and 10 μCi of ^{14}C-amino acids (specific activity 0.660 mCi mmol^{-1}) intraperitoneally 30 min before killing. The livers were removed rapidly and homogenised in 5 ml of 250 mM sucrose. RNA and protein synthesis were measured as incorporation of ^{3}H-orotic acid and ^{14}C amino acid respectively into the acid insoluble material of the liver homogenates. The results are expressed in c.p.m. per g of wet liver, each value representing the mean obtained with four animals. Figures in parentheses indicate the percentage of incorporation into the livers of the inoculated mice compared to the incorporation into the livers of the untreated animals.

The model described here should be of use in the study of the mechanisms responsible for the shutoff phenomenon and cell necrosis during lethal virus infection. Since non-replicative viral material can be highly toxic, the part played by the competition for essential metabolites as a factor in cytopathogenicity can in this case be ruled out.

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Granulomatous hypersensitivity induced by sensory peripheral nerve

THE nature of granulomas remains uncertain. In sarcoidosis and Crohn's disease of the ileum, the aetiology is unknown. Even where a causative organism has been identified, as in tuberculosis and non-lepromatous leprosy, the role the mycobacteria play in the development of the granuloma is obscure[1]. There is no animal model available for the study of granulomatous hypersensitivity. But in susceptible human subjects it is possible to produce granulomas with tubercle formation by skin testing with zirconium[2] or beryllium[3]. Similarly, in sarcoidosis, granulomas with tubercles occur in patients skin-tested with Kveim antigen (sarcoid spleen or lymph node suspension). These reactions are specific for each antigen and independent of dose. Thus, chronic states of altered tissue reactivity or granulomatous hypersensitivity can be found in humans[4]. We have shown that rabbits develop skin lesions after injection with homogenates of peripheral sensory nerve[5]. Now we report that these lesions sometimes possess organised granulomas in tubercles, and that similar granulomas can be produced by skin-testing rabbits sensitised with sensory nerve, using cutaneous nerve as the challenging antigen. These results suggest that granulomas may sometimes be an autoimmune response to tissue antigens: in the case of non-lepromatous leprosy, to an antigen in peripheral nerve containing sensory fibres[5]. A specific diagnostic test for leprosy is therefore a possibility.

It is important to distinguish granulomas from foreign body and delayed-type hypersensitivity reactions, and we have used the following criteria. First, the granuloma should be organised in tubercles, with epithelioid cells located centrally, surrounded by lymphocytes. Second, the putative epithelioid cells should possess the histological features of the epithelioid cells found, for example, in zirconium-induced granulomas, that is they should be large pale staining clear cells, having prominent nucleoli, with the chromatin of large ovoid nuclei lying peripherally[1]. Third, it should be possible to produce the granuloma by challenge with antigen alone at sites remote from the original site of injection of antigen plus Freund's adjuvant, that is a state of granulomatous hypersensitivity should exist[4]. Fourth, electron microscopy of the cells should show evidence of secretory activity, with abundant rough endoplasmic reticulum in the cytoplasm, to distinguish between the secretory epithelioid cells and macrophages[6].

Skin patches developed systemically in 14 out of 20 Dutch bantam rabbits injected with sural nerve plus Freund's adjuvant as described previously[5]. It is important to stress that none of these patches developed at the site of the injections. In four of the responding rabbits histological features of granuloma were found in the patches which had formed on the dorsum of the foot, the footpad of which had been the site of the original injection. Tubercles and developing tubercles with centrally placed epithelioid cells surrounded by lymphocytes could be seen. Four rabbits given Freund's adjuvant alone and observed for 7 months did not develop these skin patches.

A second group of rabbits was used for the skin testing experiments. Initial sensitisation was by injection into one hind footpad of 0.2–0.4 ml of a homogenate of human peripheral nerve plus Freund's complete adjuvant, prepared as described previously[7]. Three different types of peripheral nerve were tried, sural (cutaneous), dorsal roots (sensory fibres) and ventral roots (mainly motor fibres). Subsequent skin testing in all cases was with 0.2 ml 10% human sural nerve homogenised in saline and injected intradermally into the back. The sensitisation and challenge regimen is summarised in Table 1.

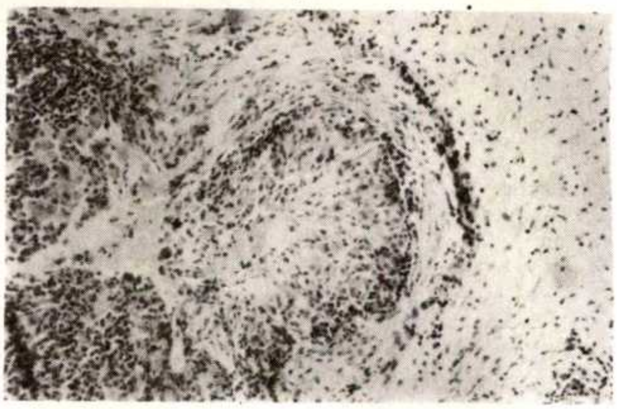

Fig. 1 Section of papule 5 weeks after intradermal injection of 0.2 ml 10% human sural nerve into back of rabbit, showing tubercule formation with epithelioid cells surrounded by lymphocytes. Stained with haemotoxylin and eosin, ×92. The rabbit had been injected with 0.2 ml sural nerve with Freund's adjuvant in the hind footpad 18 months before skin test.

Papules (5–10 mm in diameter) appeared in seven out of 18 rabbits skin-tested and these papules persisted until biopsy at 5 weeks. In the negative cases swellings were transient, not lasting beyond 2 weeks. In six out of the seven animals which developed papules, histological features of granulomas were found, that is tubercles and developing tubercles with centrally placed epithelioid cells surrounded by lymphocytes could be seen (Fig. 1). The epithelioid cells were large and pale staining with the chromatin lying peripherally in the large ovoid nuclei. Prominent nucleoli were frequently present. Langhan's-type giant cells with peripherally situated nuclei were present, the nuclei being similar in form to those of epithelioid cells[8] (Fig. 2).

In two rabbits part of the biopsy specimen was fixed for electron microscopy. In both specimens typical epithelioid cells were found, that is the cytoplasm contained abundant dilated rough endoplasmic reticulum with a moderately dense secretory product; an active Golgi apparatus was also present (Fig. 3). These observations confirm the secretory nature of the cells.

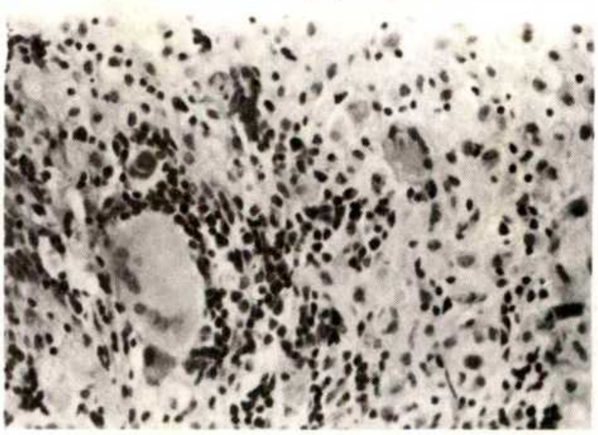

Fig. 2 Part of a developing tubercule with Langhan's-type giant cells and typical epithelioid cells, ×250. The rabbit had been injected with 0.2 ml dorsal roots with Freund's adjuvant 14 and 13 months before the skin test with sural nerve.

Rabbits which failed to develop papules showed histologically only diffuse collections of cells with no organisation into tubercles or distinguishing features of epithelioid

Table 1 Granuloma induction with various types of human peripheral nerve as sensitising antigen

No. of rabbits	Sensitising antigen	Sensitisation schedule before challenge with 10% sural antigen	Animals showing granuloma
7	Sural	A single injection (0.2 ml) 13 months to 2½ yr	4
5	Dorsal roots	14 months 0.2 ml* 13 months 0.2 ml 11 months 0.2 ml 3 months 0.4 ml	2 (1*)
4	Ventral roots	11 months 0.2 ml 10 months 0.4 ml 3 months 0.4 ml	0
2	None	—	0

*One positive had only two injections at 14 and 13 months before challenge.

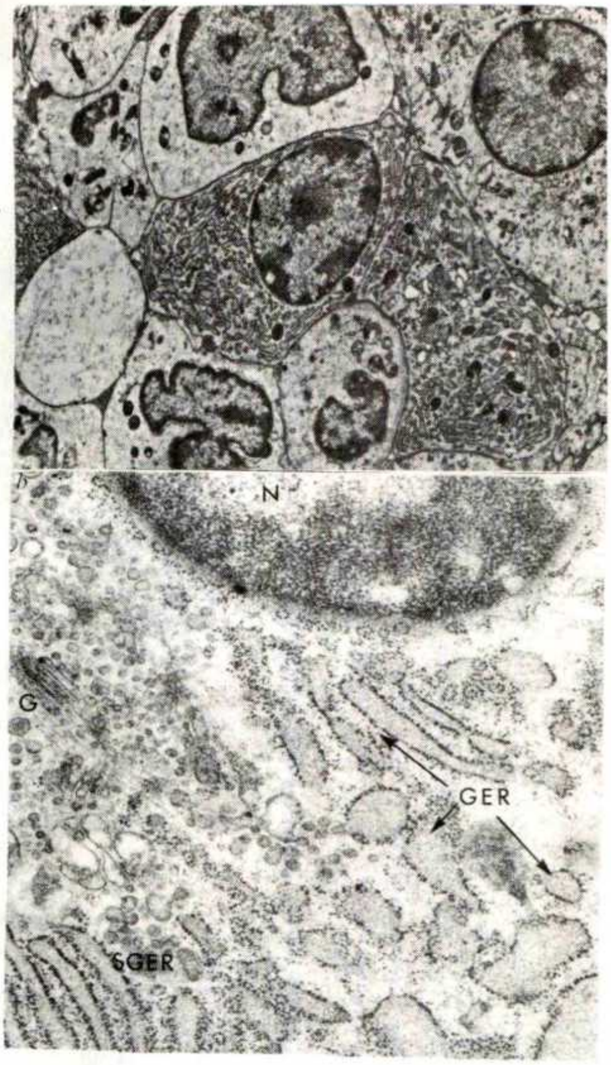

Fig. 3 *a*, Electron micrograph of part of a tubercule showing fully developed secretory epithelioid cells and undifferentiated mononuclear cells. The tubercule was taken from a skin papule 5 weeks after skin-testing with 0.2 ml 10% sural nerve in saline intradermally into the back of the rabbit previously injected with 0.2 ml sural nerve in Freund's adjuvant in footpad 2.5 yr before. Stain was lead uranyl acetate, ×3,440. *b*, Electron micrograph of part of a cell possessing the characteristic organelles of a secretory epithelioid cell. From the same tubercule as (*a*). GER, granular endoplasmic reticulum containing secretory product. SGER, stacks of granular endoplasmic reticulum. G, Golgi apparatus. N, nucleus. Stained with lead uranyl acetate, ×24,395.

cells. Injection of 0.2 ml 10% rat spleen or rat brain did not produce any papules in five out of six rabbits showing the positive granulomatous response to challenge with sural nerve; no histological features of granuloma were seen. A total of 32 skin tests, using rat spleen and rat brain as challenging antigen, produced no papules in these rabbits.

Central necrosis in tubercles was common in both spontaneously occurring and skin-test-induced granulomas which occasionally led to small abscess formation. Necrotic foci are common in beryllium granuloma[8,9] and in strongly reacting patients the Kveim test sometimes shows necrosis[10,11].

Thus we have induced granulomas and a state of granulomatous hypersensitivity in 10 rabbits and it seems that the antigen is located in peripheral nerves containing sensory fibres

A viable agent has been postulated as the cause of sarcoidosis and Crohn's disease, and samples of affected tissue have been inoculated into animals in an attempt to reproduce the disease[11-15]. Claims for successful attempts to reproduce Crohn's disease have not been confirmed[15]. Recently a virus has been isolated from ileal tissue affected by Crohn's disease, but this has not yet been shown to cause Crohn's granuloma. A viral agent, by modifying the cell surface antigen composition, could produce an autoimmune response.

In none of the experiments have the animals developed a state of granulomatous hypersensitivity, that is, skin testing at remote sites has not induced granuloma formation. Our results suggest that an autoimmune approach using tissue antigens from the ileum may be effective. The experimental model described here may be useful in testing drugs for use in sarcoidosis and Crohn's disease.

By inducing granulomas in rabbits we have reproduced the histological features of non-lepromatous leprosy; this supports previous work[5] suggesting that the skin lesions in the human disease are autoimmune responses to sensory peripheral nerve rather than being directly due to *Mycobacterium leprae*. It may also be possible to devise a skin test specific for leprosy, using sensory nerve as antigen. This test would be similar to the Kveim test for sarcoidosis, but would differ fundamentally from skin tests using the antigen *M. leprae* (lepromin) which are of no diagnostic value, giving foreign body reactions in both healthy people and those with non-lepromatous leprosy.

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Motor neurone sprouting induced by prolonged tetrodotoxin block of nerve action potentials

PERIPHERAL axons retain into adult life the ability to grow, and do so either if they themselves are cut or if neighbouring tissues are denervated (collateral sprouting[1]). Collateral sprouting also occurs in botulinum toxin poisoning[2], motor end plate disease of mice[3] and myasthenia gravis of man[4]. Muscle inactivity is common to these otherwise different conditions. We have therefore investigated whether inactivity in a muscle, produced by a local nerve block, will give rise to collateral sprouting. By paralysing motor nerves for a few days with a local application of tetrodotoxin (TTX) we have found that motor nerve terminals in the affected muscles do grow collateral sprouts. We conclude that muscle inactivity results in a sprouting stimulus to nerves.

TTX was used because it is a specific blocker of Na^+ conductance in nerve[5] and does not interfere with axoplasmic transport[6,7]. This is important because Diamond *et al.*[8,9] believe that interference with axoplasmic flow can lead to sprouting. Adult mice, male and female (20–30 g), were anaesthetised with ether, the right sciatic nerve was exposed and a hollow cylindrical cuff of Silastoseal B (Midland Silicones) was placed carefully around the nerve. Cuffs were 1.5–3 mm long, 2 mm in diameter with a 1-mm diameter hollow core. They were made as described by Lavoie *et al.*[6], except that the quantities of TTX and NaCl were different. In the first experiments the cuffs contained approximately 1 μg of TTX and 3 mg of NaCl. These paralysed for 24–48 h and two or three had to be used to give sufficient paralysis. In later experiments the cuffs contained about 5 μg of TTX and 0.5 mg of NaCl, and before insertion they were sprayed inside and out with poly tetrafluoroethylene (PTFE, DMC Industrial Aerosols, No. 889RB). This delayed release of TTX and allowed larger doses to be given, thus prolonging paralysis, but often the paralysis was not as profound as with the other type of cuff. Control animals were mice with cuffs containing no TTX and mice with TTX-containing cuffs which paralysed for only 1 or 2 d; some of these were coated with PTFE, others were not. The ineffective cuffs were left *in situ* for a further 3 or 4 d to bring the period when the cuffs were present up to that for animals where paralysis lasted for 3.5–7 d. Paralysis was checked twice a day by observing the movement and stance of the mice and by picking them up by the tail to see if their toes spread normally. Paralysis was not always complete but provided it was marked it was counted as successful. Different cuffs of a given batch did not necessarily paralyse to the same extent. We attributed this to the difficulty of mixing the small quantities of TTX, NaCl and silicone rubber into a uniform paste, so that different amounts of TTX were probably present.

Animals were used for the acute experiment within 24 h of the disappearance or marked diminution of paralysis. The peroneus tertius and solar muscles together with their nerves were dissected out of the right and left leg and studied *in vitro* at room temperature (normally 20 °C), perfused continuously with well oxygenated fluid of the following composition: 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5 mM $CaCl_2$, 1.24 mM HEPES, *p*H 7.4, 16.5 M glucose. Maximum isometric twitch and tetanic (50 per s) tension was recorded on stimulation of the nerve below the block. These tensions were compared with that evoked by direct stimulation of the muscle fibres (stimulus applied by two bright silver wires on either side of the muscle). The direct stimulus intensity was turned up gradually and usually maximum tension was produced by 10-V, 100-μs pulses. A 20-V, 300-μs pulse was then used to check that all the muscle fibres were being stimulated. If the direct and nerve-evoked tensions were not identical, the muscle was discarded as some axons had presumably been killed. This occurred on four out of 13 occasions in peroneus tertius, but not at all in 13 soleus muscles. When the two tensions were identical, a further check for absence of denervation was to grade the stimulus to the muscle nerve and, by sequentially bringing in motor units of different threshold, to count them. The number counted in this way in the experimental soleus and peroneus tertius was then compared with the figure for normal muscles from the opposite leg. When the direct tension equalled the nerve evoked tension, the unit numbers counted in this way were the same on the normal and experimental sides. The mean unit number for peroneus tertius was 11 and for soleus 21. The muscles were then curarised and end plate potentials were recorded with conventional 10 MΩ glass micropipettes. We sometimes had to use up to five times the normal dose $(5\times0.5\,\mu\mathrm{g\,ml}^{-1})$ of *d*-tubocurarine to block transmission in the experimental muscles. The recording of end plate potentials enabled us to check first that all fibres were innervated and second, by grading the stimulus to the nerve, whether as a result of sprouting any fibres had become polyneuronally innervated. Twenty to fifty fibres were examined in each muscle. Finally the preparations were stained for 5–6 h in a 4 : 1 solution of zinc iodide and osmium tetroxide[10], washed in water, teased with forceps and scissors into fine bundles, dehydrated, cleared in xylol and mounted in Permount.

Provided paralysis had lasted for 3.5 d or longer the histology showed fine ultraterminal sprouts extending from the motor nerve terminals. In some cases the fine branches were very short (Fig. 1*b*) and in others extensive outgrowths could be seen (Fig. 1*c*). The soleus endplates sprouted more vigorously than those in peroneus tertius which usually had only a single large sprout. This is in agreement with results from sprouting induced by botulinum toxin[11]. Five control animals with non-paralysing cuffs had normal endplates (Fig. 1*a*). To obtain an objective figure for the degree of sprouting, we measured for 50 endplates in each muscle, first the length, in the long axis of the muscle fibre, of the nerve

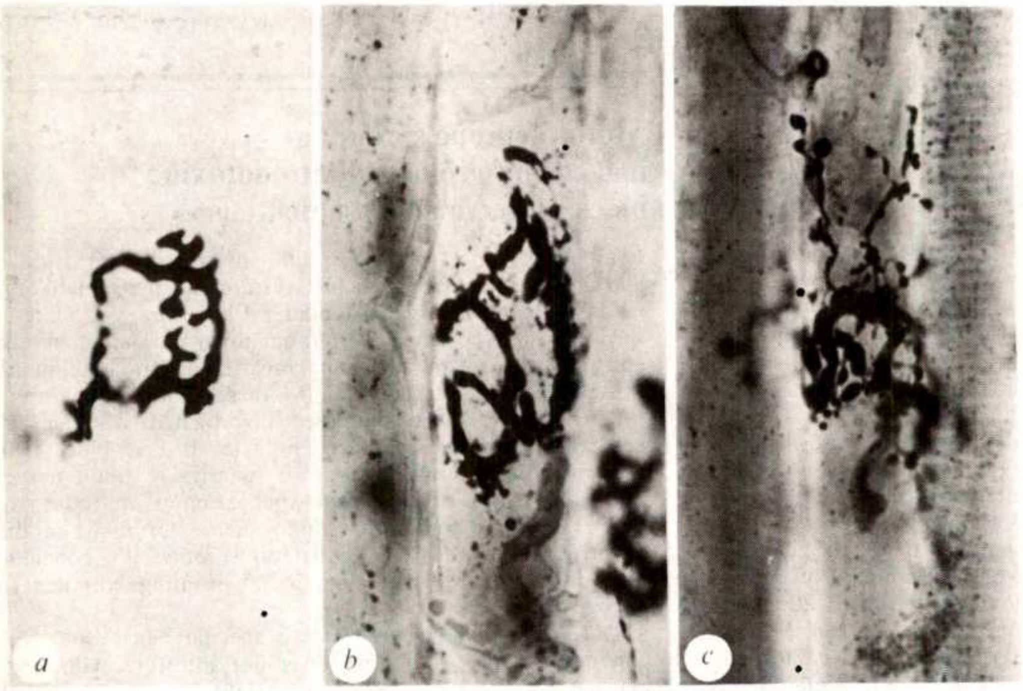

Fig. 1 Photomicrographs of mouse soleus endplates stained with zinc odide and osmium tetroxide[7] from: *a*, a control animal in which a nonparalysing cuff was present for 5 d; *b*, paralysis for 3.5d; *c*, paralysis for 5 d. The calibration bar on the right is 50 μm.

terminal which is densely stained by zinc iodide and osmium tetroxide, and second in those endplates with sprouts the length of endplate plus the furthest sprout extensions in both directions along the muscle fibre. Sprout length was taken as the difference between these two measurements. The percentage of endplates with sprouts in each experiment is shown in Fig. 2*a*, for paralysed, cuffed but not paralysed, and for normal muscles. Figure 2*b* shows, for each experimental muscle, the extent of sprouting (μm per endplate) obtained by dividing the total length of sprouts measured for that muscle by the number of endplates measured. For the control muscles the figure is near zero. As described by Barker and Ip[12], a small amount of sprouting is seen in normal muscles.

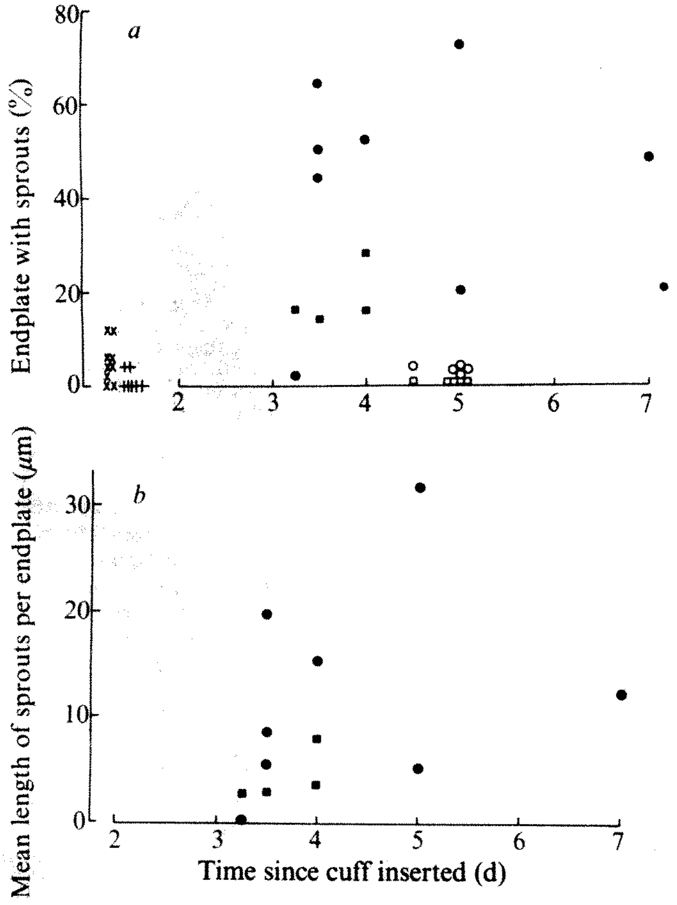

Fig. 2 *a*, Percentage of endplates with ultraterminal sprouts; *b*, mean length of sprouts per endplate (see text); ●, paralysed soleus; ■, paralysed peroneus tertius; ○, control soleus; □, control peroneus tertius; ×, normal soleus; +, normal peroneus tertius.

Even in muscles with much sprouting some endplates appeared quite normal. Our test for paralysis was not very sophisticated and it seems likely that not all the motor units were blocked for all the time, and it may also be that within one muscle the latent period before sprouting starts differs between motor units, as it does between units in different muscles[11]. Less than 2% of the fibres were found to be polyneuronally innervated by our physiological test. As a similarly very small proportion of normal mouse soleus fibres is polyneuronally innervated, it seems that the blocking times we have achieved are not long enough for sprouts to form functional endings on other fibres in significant numbers. If we can achieve longer blocks in future it will be interesting to see if they do and what their fate will be when paralysis wears off. In both normal and experimental muscles we failed to record endplate potentials in about 3% of fibres impaled.

These results show, in agreement with others[6,7] that TTX can be used to paralyse without killing nerves, and as control animals with cuffs showed no more than normal sprouting[12] any interference of the cuff *per se* with axoplasmic transport was not enough to cause sprouting. We therefore conclude that a few days inactivity of muscle gives rise to a sprouting stimulus. The work of Lomo[13,14] *et al.* has shown that inactivity induced by a local anaesthetic nerve block causes changes in the muscle membrane similar to those of denervation and this result has been confirmed by using TTX block[6,7]. The denervation changes can be prevented by direct stimulation of the muscle[13,14]. We have found (unpublished observations with G. M. Goodwin) that induction of sprouting by botulinum toxin can be prevented by direct stimulation of the muscle. Inflammation also causes muscle membrane hypersensitivity and can cause nerve sprouting[15]. It seems that sprouting is induced by a stimulus from the muscle which may be present normally to a small degree, but is greatly increased in various conditions; for example during prolonged inactivity or inflammation. Whether the stimulus is a diffusible chemical, or a change in the surface of tissues surrounding the nerve terminals is unknown.

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Punishment learning and self-mutilation in Lesch–Nyhan disease

THE self-injurious behaviours (SIB) of Lesch–Nyhan children were studied during three learning paradigms: punishment, positive reinforcement and time-out from reinforcement following an attempt at self-injury. Punishment of self-injury by contingent electric finger shock failed to suppress these behaviours but positive reinforcement of non-self injury and time-out procedures were effective. The results stress the importance of environmental factors in the development and control of SIB and suggest the presence of an unusual learning defect which may be related to the specific enzyme deficiency in Lesch–Nyhan disease.

Lesch–Nyhan disease is an autosomal recessive disease in which the primary defect is in the synthesis of a catalytically ineffective enzyme, hypoxanthine phosphoribosyltransferase[1–3]. A most striking aspect of this disease is the bizarre pattern of self-mutilation that appears at about two years of age. Finger biting is characteristic. This may cause severe injury, even amputation. Elbow splints and hand bandages may have to be worn continuously to prevent damage. If the restraints are removed, the patient screams in apparent panic, 'compulsively' raises his hand to his mouth and will bite his fingers. Once securely restrained, the patient again becomes placid. Lip biting may also develop, requiring extraction of teeth. It should be noted that the children do not appear to be deficient in pain sensitivity, and are less retarded than originally thought[4]. Many biochemical approaches to treatment have been tried without success[5].

Five males, ages 3, 5, 11, 12 and 13 yr, with the characteristic clinical and biochemical findings of Lesch–Nyhan disease served as subjects. Before treatment all wore arm restraints and exhibited scars on fingers and lips. A standardised assessment procedure was devised to establish a baseline rate of SIB while avoiding extensive injury to the child. At the beginning of the baseline period the child is seated in a wheelchair and his arms are released from the restraints (a splint that prevents the elbows from bending). An assistant seated in front of the child intercepts the hand as it nears the mouth and places it back in the child's lap without speaking or otherwise interacting with the child (response prevention). Observers out of the child's line of vision record the behaviour. For a summary of the treatment procedure and results see Fig. 1.

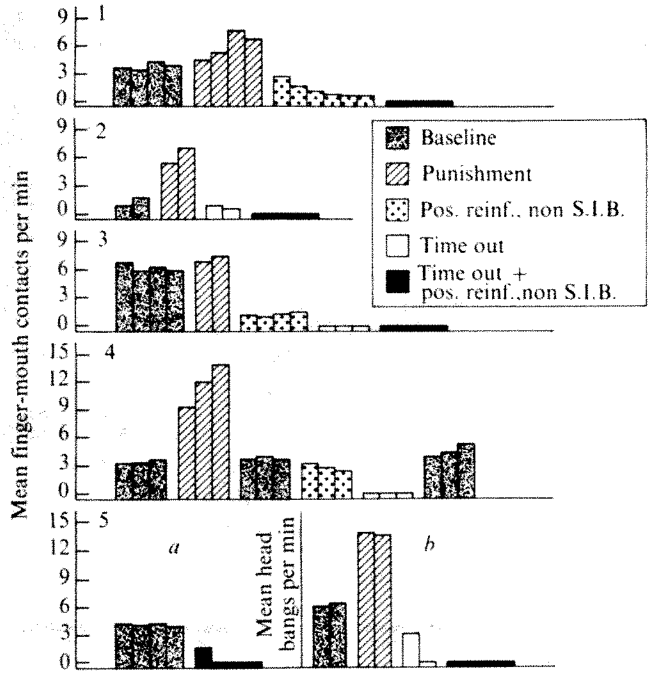

Fig. 1 Response of five Lesch–Nyhan children to treatment for SIB. Punishment does not decrease the frequency of undesirable incidents. Positive reinforcement of non-self injurious behaviour reduces the frequency; the best results are achieved with time-out and time-out plus positive reinforcement of non-SIB. Each bar represents the mean average SIB response rate per min during a session about 30 min long. Usually three sessions were conducted per day. The children's data are presented in the order of admission to the hospital.

'Aversive stimulation' has been the most widely used approach to the control of self-injury[6–12]. In the punishment paradigm, aversive stimulation is delivered immediately following the undesirable behaviour. Consent of the Human Research Committee was obtained and parents experienced the electric shock before agreeing to the treatment plan.

Subjects 1–4 were given electric finger shock contingent on each hand-to-mouth contact. The fifth child was given shock following each head bang. In all cases the shock intensity was 5 mA (a level generally described as quite painful). The results of the punishment procedure can be seen in Fig. 1. In all cases the procedure failed to suppress the frequency of SIB and, in fact, seemed to promote such behaviour.

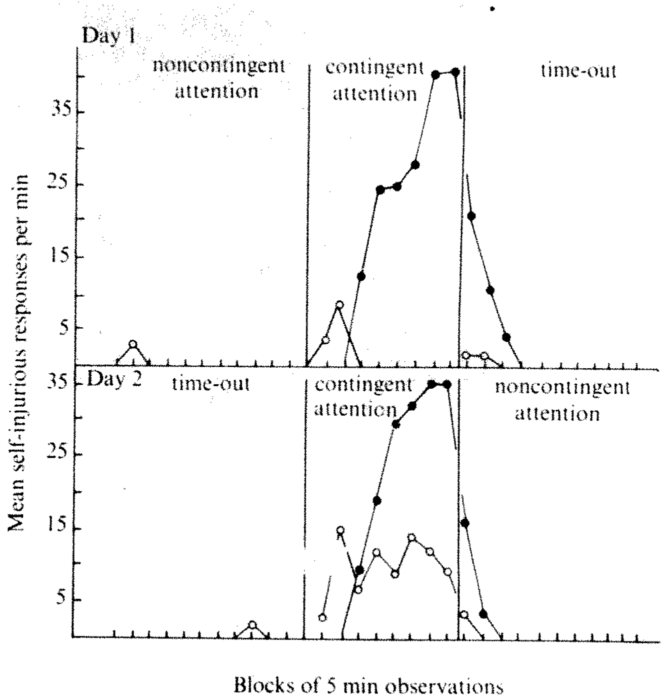

Fig. 2 SIB rate in response to non-contingent attention; contingent attention; and time-out. Each data point represents the mean number of finger biting (○) or lip biting (●) responses per min, for blocks of 5 min observations. For each of the two days the session was continuous (3 h) with no pause between manipulations.

Through naturalistic observation of parents and child it was determined that the frequency of SIB varies considerably and predictably with variations in the environment suggesting a learned behaviour that is under discriminatory control.

The facilitory influence of positive reinforcement of SIB can also be clearly demonstrated under controlled conditions. The therapist was instructed to give contingent attention by quickly interceding to prevent self-injury while making reassuring statements, and stroking the child. The frequency of SIB, in one child so treated, increased dramatically when compared to non-contingent attention or time-out (Fig. 2).

Positive reinforcement of non-SIB and time-out, either alone or in combination consistently produced rapid reductions and eliminations of SIB. When the child was not agitated or attempting to bite, the therapist would reinforce these behaviours by smiling, talking, playing or in other ways interacting with the child. Time-out consisted

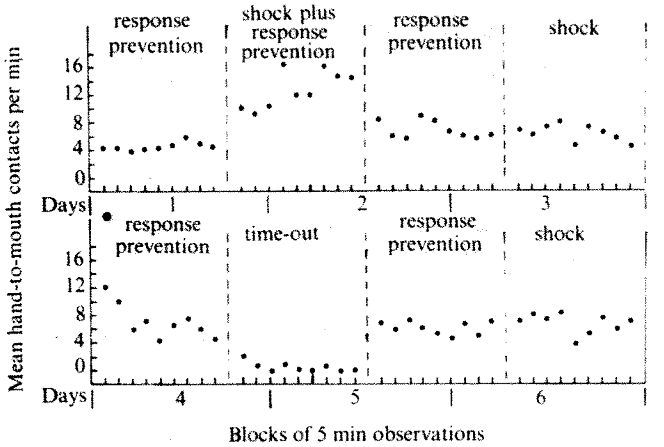

Fig. 3 Response of SIB to response interruption or baseline procedure; shock plus response interruption; shock alone; and time-out. Shock plus response interruption increases the SIB above baseline level. Shock alone produces no variation from baseline. Time-out reduces SIB to near zero. Each data point represents the mean number of hand-to-mouth contacts per min per 5 min observation period.

of the withdrawal of all attention to the child following each SIB attempt. The therapist simply turned away from the child for about 5 s. The response prevention procedure used during baseline of intercepting hand-to-mouth contacts was discontinued.

Because of the danger of self-injury, finger shock and the response prevention procedure always occurred together. This pairing of physical attention with finger shock could account for the increase in response rate during punishment. Before therapeutic training subject 5 was used to evaluate these two variable (Fig. 3). When shock is given without response interruption the SIB rate does not vary from baseline rates.

We have not shown in Fig. 1 the results of a programme for the maintenance of these effects. For all children, the rate of SIB remained at zero for approximately 3 months. At an 18-month follow-up the average SIB rate is 20% of initial baseline. Presently the behaviour is under a high degree of stimulus control with certain adults or specific settings producing complete absence of self-injury, while others produce SIB comparable to pretreatment rates.

An unexpected observation in these studies has been the inability to reduce or eliminate a particular behaviour by the administration of an aversive stimulus via the positive punishment paradigm[6]. This techniuqe is so regularly effective under experimental and natural conditions, and consistently effective in the elimination of SIB in other diagnoses, that its ineffectiveness in Lesch–Nyhan disease was particularly striking.

One explanation is that Lesch–Nyhan children are incapable of learning from aversive stimulation. In contrast, they learn quickly from positive reinforcement and time-out, suggesting a specific learning disability related to an identifiable gene. Such an explanation will depend on whether or not the inability to learn from aversive experience is specific to SIB or a general effect that extends to other aspects of learned behaviour.

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Peri-ovulatory synchronisation of behaviour in male and female rhesus monkeys

RHESUS monkeys, like many other primates, do not restrict their sexual activity to the ovulatory period, and copulation may occur at any time during the menstrual cycle[1,2]. Thus, in the laboratory, the male's ejaculatory frequency is generally highest near mid-cycle (although different pairs show different patterns of mounting and ejaculation[3]) while, in the wild, sexual activity seems to be more or less restricted to the middle part of the cycle[4,5]. It is difficult to judge the stage of the cycle in troops of feral rhesus monkeys, but during the period of maximum sexual activity females actively approach males and form temporary consort bonds which involve mutual grooming and frequent copulations[6]. To obtain an independent and objective measure of female sexual motivation, we have used an operant conditioning technique in which the female was required to press a lever for access to her male partner. This has made it possible to relate changes in female motivation precisely to changes in the circulating levels of ovarian hormones. We have also demonstrated a synchronisation and maximisation of female sexual motivation with male ejaculatory performance during the peri-ovulatory (fertile) period of the menstrual cycle.

When pairs of rhesus monkeys are tested together in laboratory conditions, the close proximity and dominance of the male, nearly twice the size of the female in this species, strongly influences the female's behaviour, and it is difficult to obtain an independent measure of the female's sexual motivation uncontaminated by the threat of male aggression. Our operant conditioning technique was developed to avoid this problem by giving the females themselves control over their access to males[7]. The testing cages were divided by a movable partition, and pressing a lever 250 times on one side of the partition activated a servo-motor that raised it, facilitating access to the other side of the cage. Females were trained to perform this task, first for food reward and then, when they were familiar with the situation, for a male partner. The period from the introduction of the female into the cage to the moment the door opened (access time) was timed automatically. After the female obtained access to the male, a behaviour test of 1 h was conducted during which observers scored various aspects of the pair's behaviour from behind a one-way vision mirror[8]. If the female failed to obtain access in the 30 min allowed, animals were removed from the operant cage and, later the same day, were brought together in a different cage for a 1-h behaviour test; this preserved the continuity of the behavioural data.

Sixteen rhesus monkeys (nine females and seven males) were obtained directly from India. The Fallopian tubes of females were ligatured to prevent pregnancy. Results are based on 1,440 tests of 17 pairs conducted during 63 menstrual cycles (30.1±4.8 d, mean±s.d.). Data were combined by aligning the cycles on the first day of the next menstruation and calculating 3-d means. The frequency of ejaculation in these pairs followed a pattern similar to that found in earlier studies[2], and showed a maximum (1.63±0.05 ejaculations per test, mean±s.e.) at the end of the first half of the cycle (reverse days 18–16), and a minimum (0.80±0.07 ejaculations per test) in the middle of the second half of the cycle (reverse days 9–7) (Fig. 1). The shortest mean access time (342.3±23.3 s) also occurred during reverse cycle days 18–16, and the longest mean access times occurred near the beginning and end of the cycle. The highest ejaculation frequency and the fastest access time therefore occurred at, or just before, the expected time of ovulation[9]. These data confirmed the preliminary results obtained with this operant conditioning technique[7]. Although the nature of the stimulus that motivated females to lever-press for access to males remains unknown, and may well vary from pair to pair, access times for males were not correlated with access times for food rewards on the same day or with access times when a female instead of a male was the reinforcement. Thus, we can assume that the access time data reflected the female's motivation for the male and were not merely dependent on cyclic changes in the female's motor activity.

To obtain more precise information on the temporal relationship between behavioural parameters and hormonal status, blood samples were collected from five females on alternate days (daily near mid-cycle) during 33 menstrual cycles. These samples were analysed for progesterone, oestradiol, testosterone and dihydrotestosterone simultaneously using a radioimmunoassay that involved chromatography on Sephadex LH20. All these cycles appeared normal, with the characteristic pre-ovulatory oestradiol peak occurring 19–17 d before the next menstruation, and with rising progesterone titres signalling the development of corpora lutea. Hormonal and behavioural data from the periovulatory periods of these 33 cycles were then combined by aligning them on the days of the oestradiol peaks (Fig. 2). The highest mean ejaculation frequency and the fastest mean access time both occurred together 1 d after the oestradiol peak, and on that day all males always succeeded in ejaculating and all females always pressed for access to their partners. The statistical analysis of the behavioural data (for cyclicity and synchrony) is necessarily complex and involves the treatment of 18 separate indices for each of the 17 pairs; results will be fully reported elsewhere.

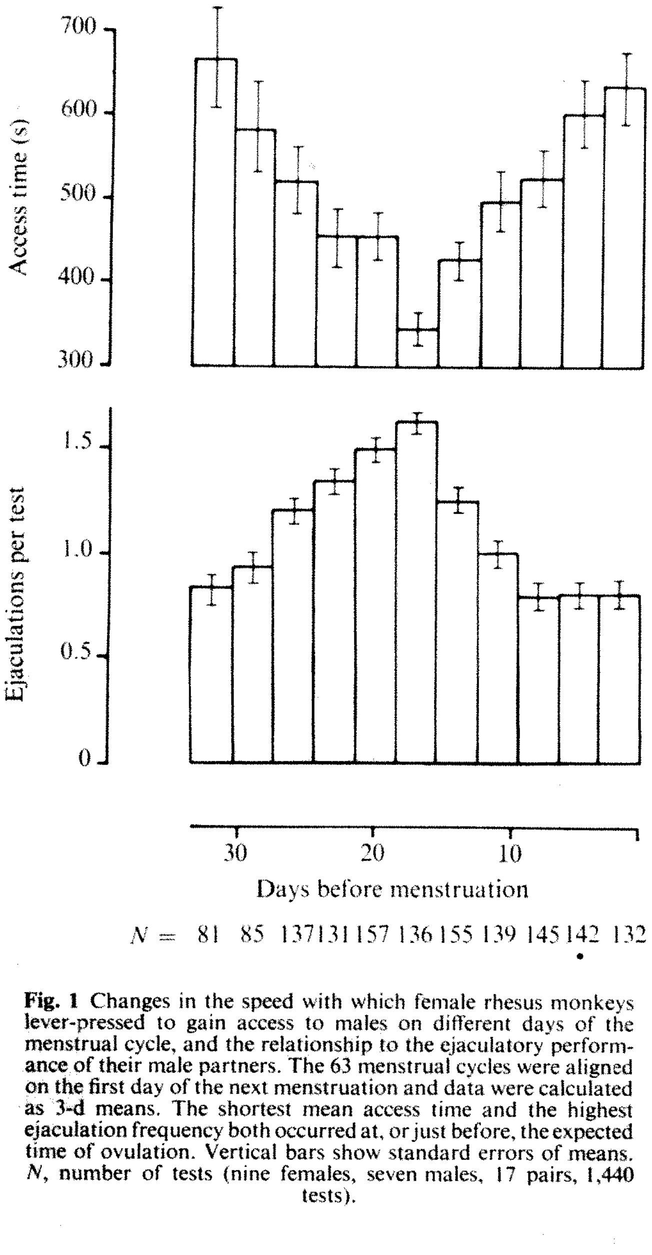

Fig. 1 Changes in the speed with which female rhesus monkeys lever-pressed to gain access to males on different days of the menstrual cycle, and the relationship to the ejaculatory performance of their male partners. The 63 menstrual cycles were aligned on the first day of the next menstruation and data were calculated as 3-d means. The shortest mean access time and the highest ejaculation frequency both occurred at, or just before, the expected time of ovulation. Vertical bars show standard errors of means. *N*, number of tests (nine females, seven males, 17 pairs, 1,440 tests).

Studies in which gonadal steroids were administered to ovariectomised rhesus monkeys showed that changes in the sexual behaviour of the pair can be induced by ovarian hormones[3]. Thus, the mid-cycle peak in plasma oestradiol, besides triggering the gonadotrophin surge responsible for ovulation 24–48 h later[10], may also synchronise and maximise the female's sexual motivation and the male's ejaculatory performance. The neuroendocrine mechanisms underlying the behavioural synchrony between the sexes and the timing of ovulation clearly have a selective advantage by optimising the chances for successful fertilisation and, thus, for survival in this highly evolved primate species in which sexual behaviour serves more than a purely reproductive function.

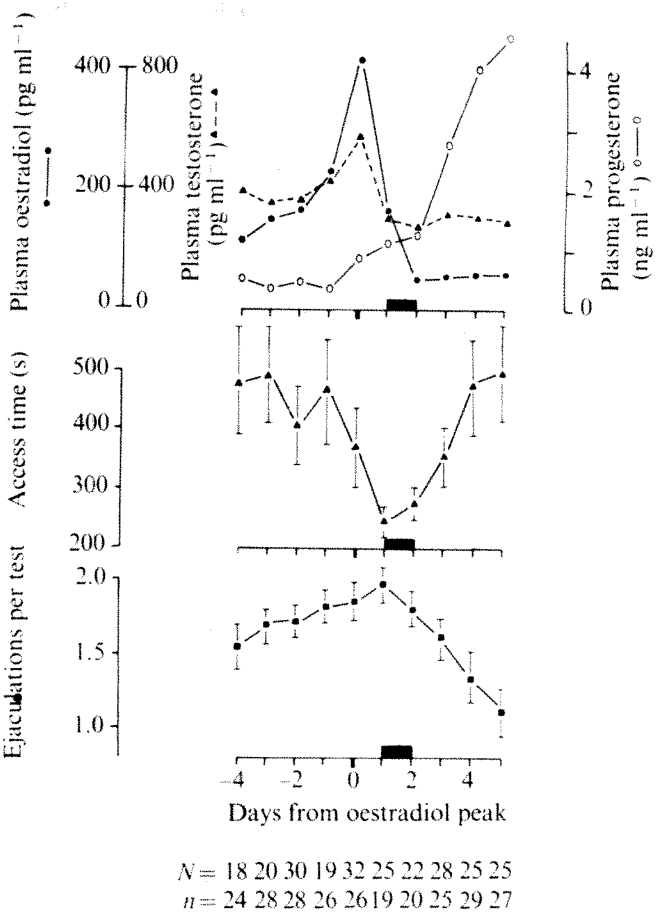

Fig. 2 Changes in the plasma steroids (oestradiol, progesterone and testosterone) of female rhesus monkeys in the peri-ovulatory period. Changes in the females' access times were synchronised with the expected time of ovulation (solid horizontal bars) when the males' ejaculations were at their maximum. The 33 cycles were aligned on the day of the oestradiol peak (day 0). Vertical bars show standard errors of means. *N*, number of plasma samples; *n*, number of behaviour tests (five females, five males, nine pairs, 252 tests).

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Ca^{2+}-dependent ATPase activity of bovine receptor cell outer segment

THE bleaching of as little as one molecule of rhodopsin can trigger hyperpolarisation of the vertebrate visual receptor cell[1]. A light modulated Ca^{2+} flux may function in the receptor cell outer segments (OS) as an intracellular messenger amplifying and transducing the photochemical signal from its disk location to the external plasma membrane[2,3]. In support of this hypothesis, Ca^{2+} has been shown to be concentrated in dark adapted disks[4] and to be released on bleaching[5–7]. This suggests the presence of a Ca^{2+}-dependent ATPase, an enzyme often associated with cellular Ca^{2+} pumps. The report that either the bleaching of a few per cent of the rhodopsin molecules within a frog rod suspension or the intra-OS elevation of Ca^{2+} to 10^{-7} M or greater, by means of ionophore A23187, can induce a rapid and very large decrease in ATP content[8] provides indirect evidence for the presence of a Ca^{2+}-activated ATPase. Interpretation of this finding is difficult, however, due to other known light-activated, ATP-dependent OS functions[9]. We present here direct evidence that purified bovine OS exhibit significant Ca^{2+}-dependent ATPase activity, which seems to be independent of contributions from subcellular contamination.

Crude OS (OS_1) were prepared under dim red light from dark-adapted bovine retina (Hormel, Austin, Minnesota) by a sucrose floatation technique (modified from ref. 10), and highly purified OS (OS_2) were prepared by the method of Dratz[11]. Both preparations were lyophilised and stored at −78 °C.

A Ca^{2+}-dependent ATPase was present in the retinal homogenate and in OS_1. In contrast to the Mg^{2+}dependent and Na^+, K^+-enhanced Mg^2-dependent ATPases, each of which exhibit a threefold decrease in specific activity during OS_1 purification, the specific activity of the Ca^{2+}-dependent oligomycin-insensitive system increased (Tables 1 and 2) during purification.

A major difficulty in interpreting these results is that OS preparations are likely to contain mitochondria from the ellipsoid of the receptor cell[12], and mitochondria contain a Ca^{2+}-dependent ATPase system[13]. Mitochondrial Ca^{2+}-dependent ATPase, however, is completely inhibited by oligomycin[14,15]. Significantly, while oligomycin-sensitive, Ca^{2+}-dependent ATPase activity is observed in the retinal homogenate, its specific activity decreases to a negligible amount as OS_2 are purified (Table 2). This suggests that mitochondrial contaminants contribute little activity, if any, to the total OS_2 Ca^{2+}-dependent ATPase activity.

Table 2 Retinal Ca^{2+} dependent ATPase activity

Sample	10 μg oligomycin	No oligomycin
Retinal homogenate	5.9 ± 0.1(2)	8.8 ± 0.5(2)
Crude OS_1	6.8 ± 0.6(2)	7.5 ± 0.1(2)
Pure OS_2	8.0 ± 0.7(1)	7.9 ± 0.8(1)

Method of assay was as described in Table 1. Oligomycin was introduced in 25λ ethanol and samples were assayed in triplicate. Results are expressed in EU per mg protein as means ± s.e. with the number of assays in parentheses.

Maximal Ca^{2+} activation was observed at concentrations of 1 mM $CaCl_2$ or greater (Fig. 1), with no activity observed at concentrations less than 0.01 mM. Because the localisation of the ATPase and the availability of the added Ca^{2+} and ATP are unknown the Ca^{2+} concentrations could be considerably lower at the enzyme site. This might explain the high concentration required to activate the ATPase in these experiments compared with intracellular levels.

Localisation of the ATPase within the disk membrane would suggest a Ca^{2+} pump system which would account for the ability of the dark-adapted disk to accumulate Ca^{2+}.

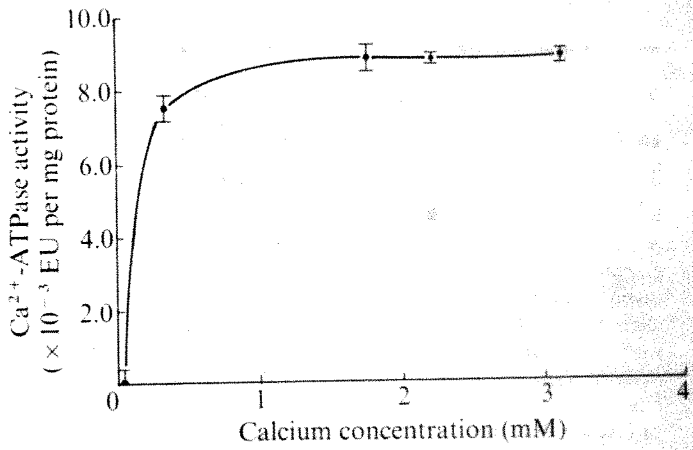

Fig. 1 ATPase activity as a function of free Ca^{2+}. Ca^{2+}-dependent ATPase was assayed as Table 1. $CaCl_2$ concentration varied. Free Ca^{2+} concentration was calculated using the association constant of the Ca $EDTA^{2-}$ salt[19].

Table 1 Retinal enzyme activities

Sample	Cyclic AMP diesterase	Mg^{2+} ATPase	Na^+ K^+ ATPase	Ca^{2+} ATPase
Retinal homogenate	7.4 ± 9(1)	29 ± 7(2)	8.8 ± 1.2(3)	5.9 ± 0.1(2)
Crude OS_1	30 ± 5(3)	9.9 ± 1.6(4)	3*	6.8 ± 0.6(2)

Cyclic nucleotide diesterase was assayed at V_{max}, using a modified two-step snake venom procedure[16]. Approximately 0.2 mg of protein was added to 1 ml containing 0.04 M Tris-HCl buffer *p*H 7.4, 0.09 M $MgCl_2$ and 0.02 M Tris cyclicAMP. The reaction was terminated after 4 min at 37 °C by a 1-min immersion in a boiling water bath. A second incubation at 37 °C with added *Crotalus atrox* venom yielded inorganic phosphate. In all assays phosphate was quantified colorimetrically by the method of Baginski *et al.*[17]. ATPase was assayed by the addition of 0.2–0.6 mg of protein to 1 ml solution containing one of the following solutions: 1, 0.1 mM EDTA, 3 mM $MgCl_2$, 0.05 M Tris-HCl buffer *p*H 7.4, 2 mM Tris-ATP, 1.35 mM Ouabain; 2, 0.1 mM EDTA, 0.15 M NaCl, 0.01 M KCl, 3 mM $MgCl_2$, 0.05 M Tris-HCL Buffer *p*H 7.4, 2 mM Tris-ATP; 3, 0.1 mM EDTA, 1 mM $CaCl_2$, 0.05 M Tris-HCl *p*H 7.4, 2 mM Tris-ATP, 10 μg oligomycin, 25λ ethanol. After a 20-min incubation at 37 °C the reaction was terminated by the addition of 4 ml of a solution containing 9% trichloroacetic acid, 1.8% ascorbic acid and 0.015% EDTA. The samples were centrifuged and an aliquot of the supernatant was removed for phosphate determination. To correct for the co-precipitation of phosphate with protein, a control containing a phosphate standard was used. The experimental details of this procedure, developed by Dr Herman Kauffman and R.A.S., will be published elsewhere. Mg^{2+}-dependent ATPase activity was obtained directly from the results of assay using mixture 1. Ouabain-sensitive (Na^+K^+-enhanced Mg^{2+}-dependent) ATPase activity was obtained by subtracting the Mg-dependent ATPase activity from that obtained with assay mixture 2. Assay mixture 3 yielded Ca^{2+}-dependent, oligomycin-insensitive ATPase activity. Protein was determined by method of Lowry *et al.*[18]. All assays were carried out in triplicate, the number of separate assays being given in parentheses. Results are expressed in EU (μmol min^{-1}) per mg protein as means ± s.e..

*The specific activity of Na^+K^+-enhanced, Mg^{2+}-dependent ATPase in purified OS was low and the enzyme assay was unstable. Assays were linear for only 5 min and the results are approximate.

Plasma membrane localisation, which cannot be excluded, would suggest a Ca^{2+}-modulated energy utilisation system, possibly involved in hyperpolarisation.

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Ultraviolet light sensitivity and delayed DNA-chain maturation in Bloom's syndrome fibroblasts

BLOOM'S syndrome (BS) is a rare, autosomal recessive condition of pre-natal and post-natal growth retardation, sun-induced telangiectatic erythema, impaired immunological function and predisposition to cancer[1,2]. BS cells show a high incidence of chromosome breakage and rearrangement often with abnormal nuclear morphology. Chromosome exchanges appear mainly to involve homologous chromosomes, while sister chromatid exchanges are abnormally frequent[3]. These features suggest a defect of DNA replication or repair. In particular, the high incidence of sister chromatid exchanges may be explained if free ends in the DNA, resulting from abnormalities of DNA synthesis and/or repair, act as initiation points for exchange[4]. We report here that BS fibroblasts are abnormally sensitive to ultraviolet irradiation *in vitro* and show a rate of DNA synthesis and DNA chain maturation lower than controls. They do not, however, have defective bypass of ultraviolet light-induced lesions during DNA replication as xeroderma pigmentosum (XP) variants[5].

Skin fibroblasts from two patients with the typical clinical and cytogenetic features of BS (H.11325/PRU8638; H.12572/PRU10003), termed BS_1 and BS_2, respectively, and normal volunteers (H.9172; H.9527) were cultured from small skin biopsies in medium TC199, supplemented with 15% human serum and 2.5% chick embryo extract and then stored in liquid nitrogen. Cells usually at the sixth to eighth and occasionally at the ninth to twelfth passage were used.

Typically, BS fibroblasts grow poorly and ours showed plating efficiency of 7–18%, while that of controls was 23–51%. The labelling index of BS cells incubated in the presence of ^{3}H-thymidine (^{3}H-Tdr) was lower than that of control cells (Table 1) and usually reached a lower plateau during a 3-d incubation (Table 2). This indicated that a larger fraction of cells was not replicating in BS than in normal cultures.

BS cultures were more sensitive to ultraviolet radiation than controls. Survival curves (Fig. 1), standardised for plating efficiency, show that the two BS strains were similarly affected and that their curves lack a shoulder. If sensitivity is expressed in terms of the dose resulting in 37% survival, the BS strains were three times as sensitive as controls.

In spite of the ultraviolet sensitivity of BS cells, our autoradiographic data (Table 3) suggest that exposure to ultraviolet light depresses DNA synthesis in control and BS cells by similar amounts. The average grain count of BS nuclei, however, was lower than that of controls (Tables 1 and 3), suggesting that the rate of overall DNA synthesis in Bs nuclei is lower than in controls even in normal conditions.

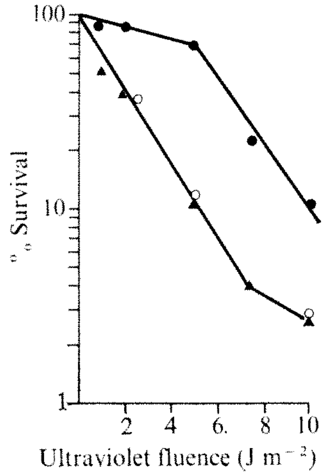

Fig. 1 Ultraviolet survival curves of normal (●) and BS (BS_1, ▲; BS_2, ○) fibroblasts. Inocula of 200 fibroblasts, plated in 60-mm plastic Petri dishes, were washed, after attachment, with phosphate-buffered saline and irradiated or sham-irradiated using a germicidal lamp emitting mainly ultraviolet light of 254 nm. When adequate colonies had grown in unirradiated cultures, all plates were collected and colonies of at least 50 cells were counted. The number of colonies scored at each ultraviolet dose is expressed as a percentage of the colonies in unirradiated cultures and shown on a logarithmic scale. The control curve was derived from eight experiments; that of BS_1 from four and that of BS_2 from one.

Pulse chase experiments showed that newly synthesised DNA from both BS cell strains consistently had lower molecular weights than DNA from control cells (Figs 2 and 3*a*). In time course experiments following a 1-h ^{3}H-Tdr pulse, this difference was maximal after a chase of 2.5 h, but persisted with diminishing magnitude when the chase was extended for up to 6 h. Ultraviolet

Table 1 Percentage of ^{3}H-Tdr-labelled cells and grain counts after 1-h pulse

	Experiment no.							
	1		2		3		4	5
Cell strain	%LC	GC	%LC	GC	%LC	GC	%LC	%LC
BS_1	5.8	40.5	5.4	41	3.2	57	16.7	2.6
Control	45	92.3	25	93	15	110	38.4	18.5

Coverslips from 60-mm Petri dishes, inoculated with 10^5 cells at the sixth to ninth passage, were collected a day later after a 1-h pulse in ^{3}H-Tr (12.5 Ci mmol^{-1}, 30 μCi ml^{-1}). They were then washed in phosphate-buffered saline (PBS), fixed in 3:1 methanol–acetic acid mixture and coated with AR 10 autoradiographic film. Autoradiographs were developed after 6-h exposure. Labelling indices (%LC) were estimated from samples of 2,000 cells and mean grain counts (GC) from 75 cells. BS cells show a 50% reduction in grain counts.

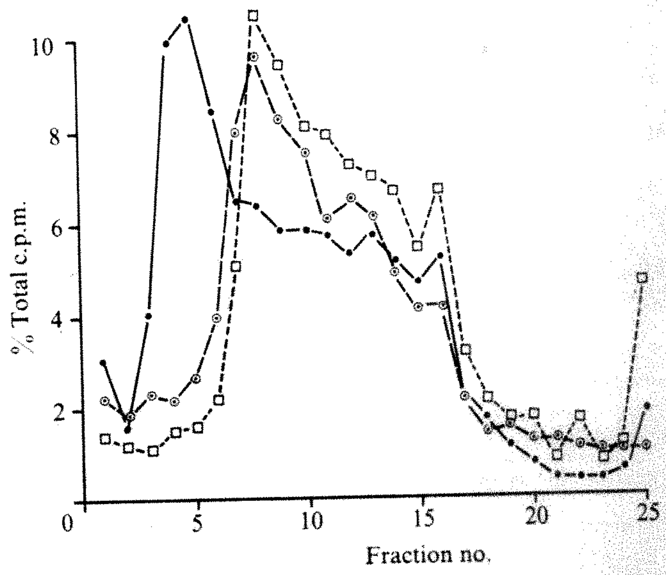

Fig. 2 Alkaline sucrose gradient profiles of newly-synthesised DNA in unirradiated control (●), BS$_1$ (□) and BS$_2$ (○) cells. 2.5 × 10^5 cells were cultured as described in the text in 60-mm Petri dishes and incubated for 2 d, washed in PBS (8 g of NaCl, 0.2 g lf KH_2PO_4, 0.2 g of KCl, 1 μg of Na_2HPO_4 per l of water) and incubated in 5 ml of warm medium for 1 h, pulse-labelled with ^{3}H-Tdr (33 μCi ml^{-1}; 25 C mmol^{-1}) for 1 h, washed with warm PBS and chased for 2.5 h with unlabelled medium containing thymidine and deoxyeytidine (both at 10μM). After incubation, cells were scraped off the Petri dishes with a piece of silicone rubber into 0.3 ml of PBS containing EDTA (0.2%), centrifuged (600g for 5 min at 18 °C) and resuspended in 0.15 ml of EDTA solution. The suspension was then exposed to δ irradiation (1.5–2.0 krad) from a ^{60}Co source to prevent entanglement of DNA strands[6]. Alkaline sucrose gradients (4.4 ml, 5–20%) containing 0.1 M NaOH and 0.1 M NaCl were overlain first with 0.2 ml of 2% sodium dodecyl sulphate containing 0.02 M EDTA; and then with 0.1 ml of the cell suspension. Centrifugation was for 75 min at 20 °C and 40,000. p.m. in an SW56 rotor in a Beckman L2-65B ultracentrifuge. After centrifugation, 10-drop fractions were collected from the bottom of the tubes and assayed for cold acid-insoluble radioactivity[7]. Sedimentation was towards the left.

irradiation before labelling (Fig. 3*b*) depressed the maturation of DNA chains to a similar extent in BS and control cells, while caffeine had no detectable effect in unirradiated control or BS cells (Fig 3*c*), but caused a moderate depression of DNA chain maturation in ultraviolet-irradiated control and BS cells (Fig. 3*d*). In contrast, and in agreement with Lehmann *et al.*[5], cells from a patient with XP-variant showed a much greater inhibition of DNA chain maturation after ultraviolet irradiation and this was accentuated by caffeine (Fig. 4). This effect of ultraviolet irradiation on XP-variant cells has been attributed to a defect in the bypass of induced lesions, probably due to a delay in the sealing of gaps left opposite pyrimidine dimers during the synthesis of new DNA strands (post-replication repair[5]). Ultraviolet irradiation with and without caffeine had no differential effect on the maturation of newly synthesised DNA of control and BS cells, suggesting that this form of repair is not inhibited in BS, as it is in XP-variants.

Our experiments show that BS fibroblasts *in vitro* have low viability and are abnormally sensitive to ultraviolet irradiation, their rate of DNA synthesis is low and maturation of new DNA chains is delayed. This is in keeping with the clinical features of BS,

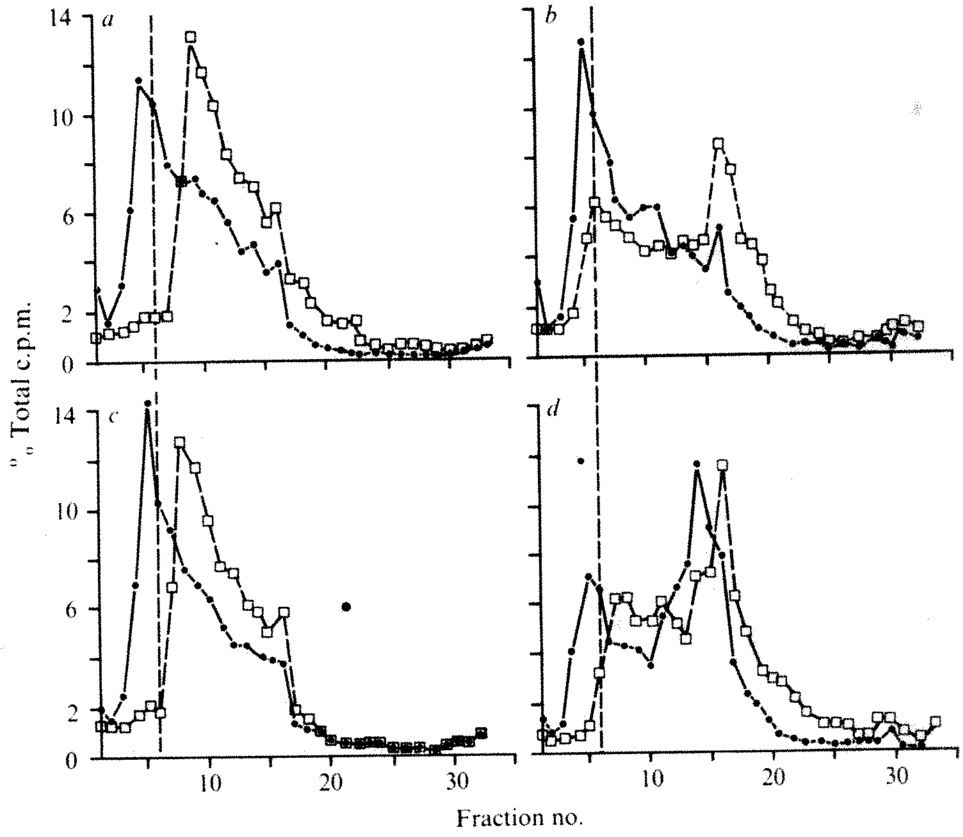

Fig. 3 Alkaline sucrose gradient profiles of DNA newly synthesised, either in the absence or presence of caffeine, by ultraviolet-irradiated and non-irradiated BS$_1$(   ) and control (   ) cells; methods were as described in Fig. 2, except for irradiated and/or caffeine-treated cells which were exposed, immediately after the initial washing with PBS, to germicidal light (mainly of 254 nm) at a fluence of 12.5 J m^{-2} and/or caffeine (0.3 mg ml^{-1}). The vertical line marks the sixth fraction of each gradient. *a*, Nonirradiated, no caffeine; *b*, Irradiated, no caffeine; *c*, non-irradiated caffeine; *d*, irradiated, caffeine. As in Fig. 2, the DNA of the BS cells sediments more slowly than the control and this difference is not influenced by the treatment, even where irradiation and caffeine result in a noticeable reduction in the sedimentation rate of both control and BS DNA (*d*).

Table 2 Percentage of ^{3}H-Tdr-labelled fibroblasts as a function of time

Cell strain	Experiment no.	Labelling period (h) 14	24	38	48	62	72
BS_1	1*	9.25	19.4	—	29.7	31.7	—
	2†	33.1	34.0	35.6	41.1	38.9	53.4
BS_2	2	55.5	69.5	74.0	74.8	75.6	78.5
	3‡	10.7	35.5	—	44.0	55.0	59.0
Control	1*	46.9	89.2	—	92.0	93.6	—
	2	59.1	66.5	72.1	71.4	69.4	74.1
	3	30.4	76	—	72	78	83

*Cells at the eight passage.
†Cells at the fifth passage.
‡Cells at the ninth passage.

Cells at similar passages were plated in 35-mm Petri dishes containing coverslips (4×10^4 per dish) and labelled with ^{3}H-Tdr (1 μCi ml^{-1}, 1 Ci mmol^{-1}). The label was replenished daily. Collected coverslips were washed with PBS, fixed in 3:1 methanol–acetic mixture and coated with AR 10 autoradiographic film. Autoradiographs were developed after 10-d exposure. Samples of 2,000 cells were used to determine labelling indices.

Table 3 Mean grain counts per labelled nucleus after 1-h ^{3}H-Tdr pulse

Cell strain	Experimental treatment: No irradiation, no caffeine Mean	s.e.	No irradiation, plus caffeine Mean	s.e.	Irradiation, no caffeine Mean	s.e.	Irradiation, plus caffeine Mean	s.e.
BS_1	119	6.3	127	8.8	39.7	2.4	50.1	2.8
Control	131	8.1	182	8.4	71.0	4.0	76.9	3.9
BS_2	125	8.1	—	—	41.5	4.2	42.7	4.7
Control	186	7.6	227	23.4	58.3	2.6	62.6	3.6

Cells were plated in Petri dishes containing coverslips and treated as for those in Fig. 3. They were collected and autoradiographs were prepared as described in Table 1. Samples of 50 cells were used to calculate the mean grain counts. **BS** cells show a 30% reduction in grain counts. This is less than observed in Table 1; such discrepancy may be due to the different experimental procedures to which the cells have been exposed.

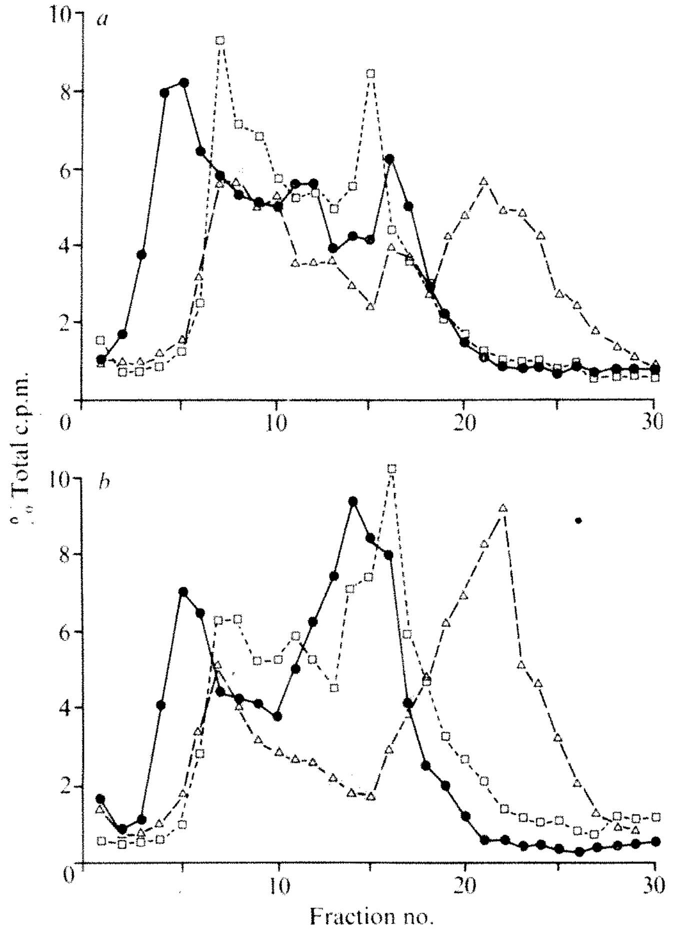

Fig. 4 Alkaline sucrose gradient profiles of DNA newly synthesised, either in the absence or presence of caffeine by ultraviolet-irradiated control (●), BS, (□) and XP variant cells (△) labelled and chased as in Figs 2 and 3. Experiments were carried out either in the absence (*a*) or presence (*b*) of caffeine (0.3 mg ml^{-1}), throughout the period after ultraviolet irradiation.

particularly growth retardation and light sensitivity. In contrast to XP-variants, when BS cells are exposed to 12.5 J m^{-2} of germicidal ultraviolet light they do not seem to have difficulties in bypassing ultraviolet-induced lesions in the course of DNA replication and they seem to respond normally to the inhibitory action of ultraviolet light on DNA synthesis.

Data of Hand and German[8] suggest that movement of DNA replicating forks is slower in BS than in control cells. By their autoradiographic technique, they could measure DNA chain growth of relatively small samples of replicons from a few replicating cells. Our sedimentation experiments, however, compare the average rate of maturation of DNA chains attaining a maximum size of 150 μm (approximately 5 replicons 30 μm long) from whole cell populations[5,9].

Our results therefore are in line with and extend those of Hand and German[8]. Because their technique depends on cellular uptake of ^{3}H-Tdr, they considered the possibility that their results reflect impaired uptake of ^{3}H-Tdr by BS fibroblasts. We excluded this possibility because, after a 2.5-h chase (during which DNA synthesis was not dependent on exogenous ^{3}H-Tdr), differences in molecular weight of BS and control DNA were even greater than immediately after the pulse (data not shown).

DNA synthesis in mammalian cells is initiated in a preprogrammed fashion at different sites of the same molecule and proceeds bilaterally from each origin[9-11]. The rate of movement of the growing fork may change during the S phase[10] and the distance between initiation points may vary between cells of the same organism according to the stage of differentiation and development[11]. Our data for BS cells suggest that the fundamental defect involves a step in DNA replication, but we cannot exclude the possibility that the impaired growth of new DNA chains is secondary to a disturbance of other metabolic processes. The abnormal sensitivity of BS fibroblasts to ultraviolet light cannot yet be explained by a defect of DNA repair, for analysis of the first steps of excision repair has given negative results[12,13] and our observations suggest that the bypass of ultraviolet lesions is not impaired as in XP-variants. The association of ultraviolet-sensitivity and delayed maturation of DNA chains in BS fibroblasts, however, suggests that the metabolic defect in BS involves a factor or step important for DNA synthesis and for the repair of ultraviolet-induced damage.

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Chromatin dispersal and DNA synthesis in G1 and G2 HeLa cell nuclei injected into *Xenopus* eggs

IF HeLa cells that are synthesising DNA (S phase) are fused with others in the G1 phase of the cell cycle, DNA synthesis is prematurely induced in the G1 nuclei. But if S-phase cells are fused with G2 phase cells, which have already replicated their DNA, synthesis is not induced in the G2 nuclei, although it continues in the S-phase nuclei[1]. Evidently nuclei of G2 cells contain components that prevent replication of their DNA even in the presence of the enzymes and substrates. If cell nuclei are injected into eggs of *Xenopus laevis* they usually swell within 1 h to many times their original volume, the chromatin becoming dispersed throughout the swollen nucleus. Simultaneously, DNA replication begins in the nuclei[2]. We have injected nuclei of G1 and G2 HeLa cells into *Xenopus* eggs in a study of the relationship between this chromatin dispersal and DNA synthesis, and the restriction to DNA synthesis in G2 nuclei.

Figure 1 shows the size distribution of G1 and G2 HeLa cell nuclei 5, 60 and 120 min after injection of ruptured HeLa cells into *Xenopus* eggs. At 5 min, before the nuclei had swollen appreciably, they were all below 17-μm diameter. Also, the distribution of G1 and G2 nuclei between the two size groups was roughly the same (although a small but significantly higher percentage of G2 nuclei than G1 were greater than 12-μm diameter ($P = 0.05\%$)). By 60 min some of the G1 and G2 nuclei had swollen above 17 μm, and by 120 min many more had done so and also many of the smallest nuclei had moved into the middle size group. At 120 min any differences in size distribution between G1 and G2 nuclei were of little significance ($P > 20\%$). Hence G1 and G2 HeLa cell nuclei swell to a similar extent in *Xenopus* egg cytoplasm.

Xenopus egg cytoplasm, however, does not induce similar rates of DNA synthesis in the nuclei of these ruptured G1 and G2 HeLa cells. Figure 2 shows the number of silver grains over sections of HeLa nuclei in *Xenopus* eggs which were fixed for autoradiography 60 min after injecting ruptured G1 and G2 HeLa cells and ^{3}H-thymidine. The number of grains over the most swollen G1 nuclei was comparable with that counted over the pronuclei of the *Xenopus* eggs, namely a mean of 26.6 over 10 pronuclei. Precise comparisons from these counts of rates of DNA synthesis between the HeLa cell nuclei and *Xenopus* pronuclei are impossible because of the different concentration of DNA per unit area of cross-section. But, because the egg pronuclei completely replicate their DNA within about 45 min of activation of the egg by pricking[2], it is clear that the largest G1 nuclei must also have replicated a large part of their DNA. As the size of the G1 nuclei decreases, the number of silver grains per nuclear section also decreases (and hence the ^{3}H-thymidine incorporated per nucleus must decrease more rapidly still, since the sections of smaller diameter contain a higher proportion of the nuclear DNA).

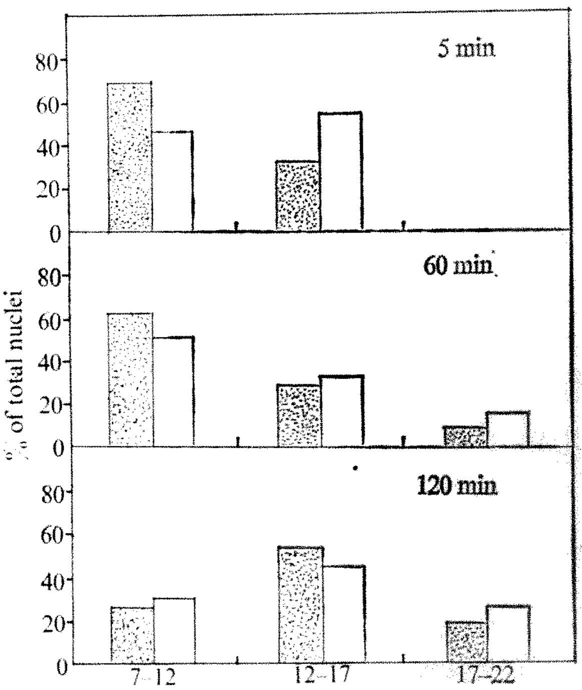

Fig. 1 Size distribution of HeLa cell nuclei at intervals after injection of ruptured HeLa cells into *Xenopus* eggs. G1, stippled; G2, plain. HeLa cells were grown as monolayers in Eagle's medium with added foetal calf serum, and synchronised by double thymidine block[3]. Cells were suspended for injection in a solution of sucrose (1.2 M), $MgCl_2$ (1.5 mM), vinblastine sulphate (0.625 mM) and Tris-HCl (1 mM) *p*H 7.2. *X. laevis* females were induced to ovulate[4], and the eggs dejellied[5]. About 20 cells were injected[6] into each egg in 50 nl of medium which also contained ^{3}H-thymidine (25 nCi, 0.4 ng Radiochemical Centre, Amersham). Vinblastine was present to retain the egg pronucleus at the animal pole. After 5, 60 or 120 min at 18 °C the eggs were fixed in a modified Perenyi's fluid and sectioned at 5 μm. Sections were mounted and subjected to autoradiography with Nuclear Emulsion L 4 (Ilford) with 7-d exposure at 0–2 °C. They were then stained with Mayer's haematoxylin and eosin. Nuclear diameters of over 100 G1 and G2 nuclei in over 20 eggs were measured at each time with a calibrated eyepiece graticule as the mean of the longest and shortest axis.

In contrast, all G2 nuclei incorporated little thymidine, so the block to DNA replication of even the most swollen G2 nuclei has largely been retained, and is effective against the replicating enzymes of the *Xenopus* egg.

In these experiments ruptured whole HeLa cells were injected into the eggs. Figure 3 shows the results of an experiment which was identical except that before injection the G1 and G2 HeLa cell nuclei were isolated by a standard procedure. The G1 nuclei incorporated roughly the same amounts of ^{3}H-thymidine as before, but the G2 nuclei incorporated almost as much as the G1, showing that their block to DNA replication has been lost.

DNA replication in the egg and sperm pronuclei after fertilisation is always accompanied by swelling of the pronuclei[7], and previous work has suggested that there is a causal connection between the two processes. Thus, when

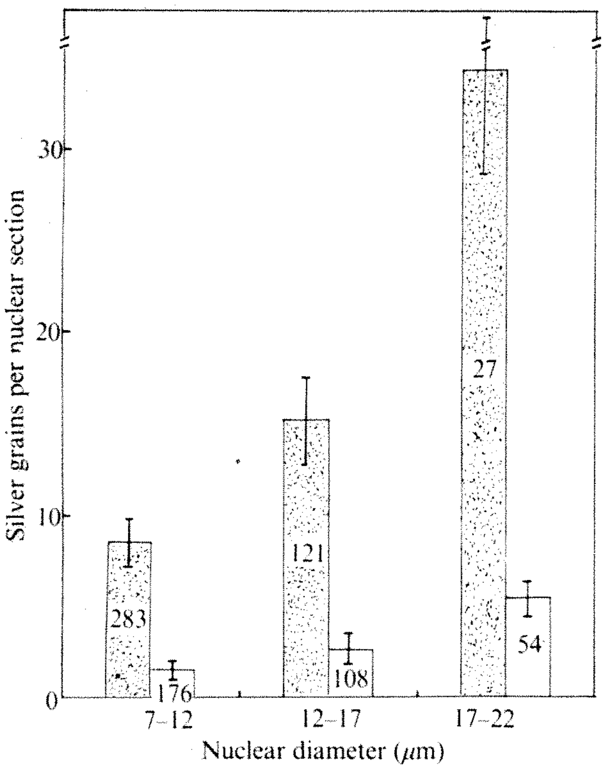

Fig. 2 ^{3}H-thymidine incorporated into nuclei of ruptured HeLa cells by 60 min after injection into *Xenopus* eggs. G1, stippled; G2, plain. The experiment was performed as described in Fig. 1. The number of silver grains per nucleus is the number over each nucleus less the number over a similar area of egg cytoplasm in the same section, values are mean ± s.e. The number of nuclei scored is shown in each column.

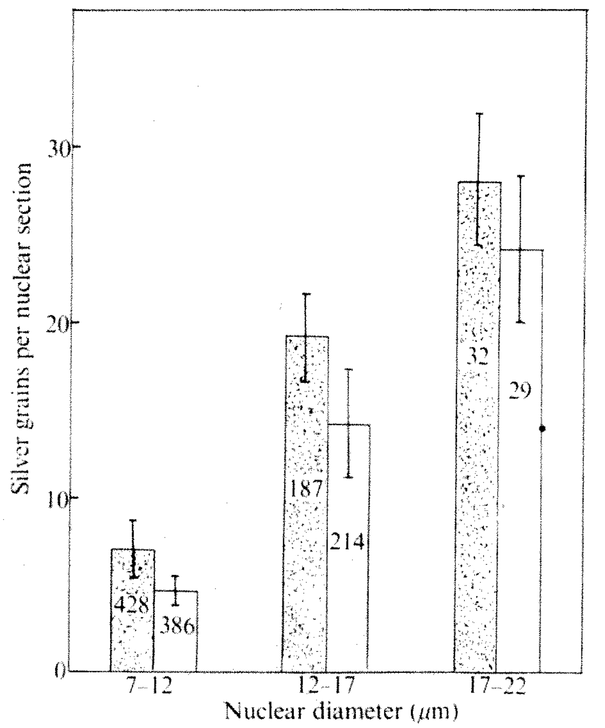

Fig. 3 ^{3}H-thymidine incorporated into isolated HeLa cell nuclei by 60 min after their injection into *Xenopus* eggs. G1, stippled; G2, plain. HeLa cells were suspended in a solution of $MgCl_2$ (3 mM) and Triton-X100 (0.1%) and, after 15 min, homogenised in a glass tube with Teflon plunger. The homogenate was centrifuged for 10 min, at 900*g*, resuspended in sucrose (0.32 M) and $MgCl_2$ (3 mM) and centrifuged again. The pellet was suspended in a little sucrose (2.1 M) and $MgCl_2$ (1.5 mM), layered on top of the same solution, and centrifuged for 1 h at 100,000*g*. The nuclei were suspended for injection as described in Figs 1 and 2. Data are mean ± s.e.

somatic cell nuclei were injected into *Xenopus* eggs, nuclei that did not incorporate ^{3}H-thymidine were little if at all swollen, and the average volume of the nuclei increased at the same rate as the proportion that replicated DNA[2]. Also, those parts of injected brain nuclei which were the first to swell were the first to synthesise DNA[3]. It has not been clearly demonstrated, however, whether swelling is an essential prerequisite for DNA replication, or merely a consequence of it. Our finding that when ruptured HeLa cells are injected into *Xenopus* eggs G1 and G2 swell to the same extent, but only G1 nuclei replicate a significant portion of their DNA, strongly suggests that swelling is not merely a consequence of DNA synthesis, but is a prerequisite for it. Nuclear swelling in the *Xenopus* egg, with its associated chromatin dispersal, seems to be dependent on a lowering of the unbound cation concentration of the egg cytoplasm to a level at which bound cations dissociate from the DNA of chromatin[4,9], but seems to be independent of the discharge of cortical granules at activation[10].

The ability to inhibit DNA replication is thus retained in the nuclei of ruptured G2 HeLa cells injected into *Xenopus* eggs, but almost completely lost if the nuclei are isolated before injection. Hence the inhibition is most probably caused by a loosely bound constituent not normally reckoned among the components of nuclei and chromatin, or by a labile modifying group attached to a known component. Alternative methods of isolating G2 HeLa cell nuclei may retain the ability to block DNA synthesis and facilitate identification of the components involved.

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Evidence for cytoplasmic control of gene expression in higher plants

ONE of the difficulties of studying regulatory gene function in higher eukaryotes is the lack of genotypes with single gene differences in constant genetic backgrounds. In the Triticinae, however, the construction of a series of aneuploids[1,2] makes it possible to monitor the activity of single gene loci in different genetic backgrounds. With these genotypes we have been able (unpublished results with T. Lelley) to localise the structural genes for phosphodiesterase (PDE, EC 3.1.4.1.) on chromosomes 3A, 3B and 3D of wheat, *Triticum aestivum* ($2n=6x=42$). We also showed that regulatory genes located on chromosomes 5A, 5B and 5D enhance the activity of the structural genes. We now report that extrachromosomal control of the PDE isoenzymes is another component of this complex regulatory system.

Reciprocal hybrids were constructed between hexaploid wheat and its diploid relative, *Secale cereale* ($2n=14$), resulting in amphidiploid derivatives with wheat cytoplasm in

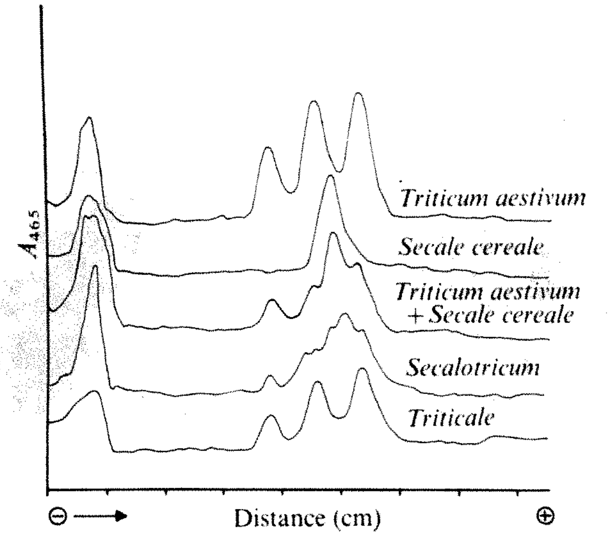

Fig. 1 Densitometer tracings of PDE isoenzyme patterns of wheat, rye, and wheat/rye hybrids. Extraction from leaves with 3 × (w/v) 0.05 M Tris-HCl, *p*H 7.5, at 4 °C. Centrifugation was at 20.000 *g* for 10 min. This crude extract was used for separation in 7% polyacrylamide gels, 0.6 × 9 cm, according to Davis[9]. The gels were stained for 5′-phosphodiesterase by incubation in 0.05 M Tris-HCl, *p*H 7.5, and 2′-deoxythymidine-5′-phosphoric acid-1-naphthylester and Fast Blue RR for 30–60 min. The gels were scanned in a Joyce–Loeble densitometer at 465 nm. (The peak in the upper part of the gels is caused by an unspecific adsorption of Fast Blue to fraction-I protein).

Triticale ($2n=8x=56$) and with rye cytoplasm in ***Secalotricum*** ($2n=8x=56$). We used several individual plants per genotype with cytologically verified chromosome numbers.

Polyacrylamide gel electrophoresis patterns of the PDE isoenzymes of both genotypes were compared with those of the parents (Fig. 1). The wheat pattern showed the well known three isoenzymes[3,4]. The slow-moving enzyme is a product of the gene on chromosome 3D (*Pde-D3*); two fast-moving isoenzymes with an identical mobility are produced by the structural genes *Pde-A3* and *Pde-B3* on chromosomes 3A and 3B, respectively, and the hybrid enzyme is composed of the product subunits of the genes *Pde-D3, Pde-A3 and Pde-B3.* Rye possesses only one PDE enzyme. Its mobility was identified by coelectrophoresis of wheat and rye extracts (Fig. 1) as being between those of the fast moving isoenzyme and hybrid enzyme of wheat. The *Secalotricum* pattern clearly shows the wheat and rye isoenzymes while the pattern of the *Triticale* with wheat cytoplasm does not exhibit the rye band. Thus the rye gene is expressed in the presence of rye cytoplasmic factors but not in the *Triticale* with wheat cytoplasm. Two additional isoenzymes in *Secalotricum* were not present in the parent species. The number, positions and dimeric nature of the PDE isoenzymes (unpublished work of G. W.) makes them likely to be hybrid enzymes composed of wheat and rye monomers (Fig. 2). Further evidence for the cytoplasm-dependent expression of the rye PDE gene came from wheat cytoplasmic wheat rye addition lines[2], where the seven rye chromosomes were added separately to the wheat genome. No difference in PDE activity was found between any of the seven addition lines and wheat or *Triticale* (not shown). Thus it is clear that the structural gene for the rye PDE enzyme is under cytoplasmic control. This gene cannot be expressed in genotypes with wheat cytoplasm. The structural and regulatory genes of wheat, however, are active in the presence of the rye cytoplasm.

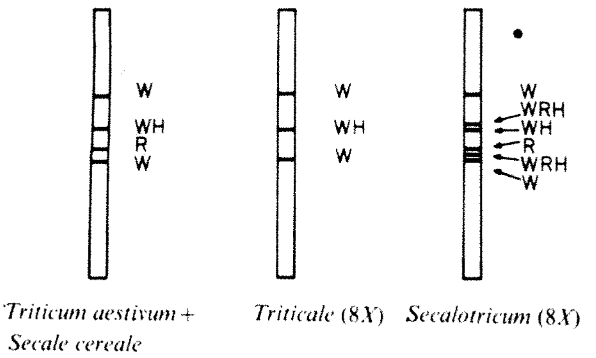

Fig. 2 Diagramatic interpretation of the PDE isoenzyme patterns in wheat/rye hybrids. W, wheat enzymes; WH, wheat 'hybrid' enzymes; R, rye enzyme; WRH, wheat/rye 'hybrid' enzymes.

In summary our studies on the genetic control of the PDE isoenzymes in the Triticinae reveal a complex system of regulation of gene expression. Our earlier studies (unpublished) showed that (1) the activity of the isoenzymes depends on the dose of the structural genes; (2) the activity of the structural genes is regulated by separate nuclear genes; (3) the effect of the regulatory units is also dose-dependent; (4) the regulatory and structural units are located on different genomes (B and D) of the allohexaploid wheat: thus, the complexity of regulation seemed to have increased with polyploidisation. We have now shown further that (5) in rye corresponding structural genes for PDE activity are under cytoplasmic control.

There have been several reports of the location of structural genes coding for enzyme proteins in wheat[5–7]. Also, cytoplasmic control of the expression of nuclear genes has been observed in cereals[8]. The effects described, however, were only quantitative and reflect a type of gene control which is well documented. Our study also demonstrates qualitative effects- the absence of isoenzymes—of cytoplasmic factors on the expression of a nuclear gene with a well defined product. The PDE isoenzymes of wheat and rye are sufficiently homologous to form hybrid enzymes (Fig. 1), and so it is surprising that the corresponding regulatory factors of the wheat and rye cytoplasm are apparently quite different in their function. The fact that the nuclear structural genes of wheat are expressed in the presence of the rye cytoplasm may be a consequence of the polyploid nature of wheat where enhancing regulatory genes are present in triplicate (for example, on chromosomes 5A, 5B and 5D, unpublished results with T. Lelley). Our results strongly suggest that there is a complex interaction of structural genes not only with nuclear regulatory factors but also with cytoplasmic control factors, which are inherited from the female parent only. We therefore suggest that future experiments on gene regulation should take into account the existence of (as in this case) strong extrachromosomal control factors, so that existing models on regulation of gene expression (see, for example, refs 10 and 11) may be extended. Our hypothesis of gene regulation can be tested further using haploid hybrids, rye cytoplasmic wheat and rye cytoplasmic addition lines which are now being constructed.

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High resolution detection of DNA–RNA hybrids *in situ* by indirect immunofluorescence

WE describe here a new method for the detection of RNA–DNA hybrids in cytological preparations with which we have revealed the locations of hybrid molecules on polytene chromosomes. The critical reagent is an antiserum raised in rabbits against poly(rA)·poly(dT) complexed with methylated bovine serum albumin, originally described by Stollar[1]. The specificity and resolving power of the indirect immunofluorescence procedure are demonstrated using *in situ* hybridisation of 5S rRNA (ribosomal RNA) to polytene chromosomes of *Drosophila melanogaster* as a model system. The method has significant advantages over the autoradiographic procedures[2–5] used so far.

The procedure for visualising the *in situ* hybrids follows Alfageme *et al.*[6] It consists of exposing the cytological preparation to the rabbit anti-hybrid antiserum, then to anti-rabbit IgG prepared in goat and tagged with rhodamine, followed by examination in a fluorescence microscope (see legend to Fig. 1). Our test objects were polytene chromosomes of *Drosophila melanogaster* (giant phenotype) to which 5S rRNA had been hybridised *in situ* (see legend to Fig. 1). The two glands from a single larva either were used as duplicate samples or one gland served as a 'control' for the other. The preparations were not air dried at any time during the procedures. Test slides which were air dried at some stage before the immunological reactions were inferior to their controls either in the morphology of the chromosomes, the background fluorescence levels, the uniformity of the fluorescence staining or a combination of defects.

In situ hybridisation followed, in general, the recipe of Pardue and Gall[7] with modification by Alonzo *et al.*[8] (see legend to Fig. 1). The reaction was effective with 1.0 μg and with 0.2 μg 5S rRNA per slide. It is likely that much smaller amounts could be used if applied in a smaller volume and, perhaps, for longer times[7,9].

The specificity of the immunological reagents in the cytological reaction is demonstrated by the confinement of chromosomal fluorescent label to the 56F region when 5S RNA is included in the *in situ* hybridisation medium (Fig. 1) and by the absence of chromosomal fluorescence when 5S rRNA is omitted (not shown; would be black). Further evidence that the anti-hybrid antibodies are responsible for the chromosomal site of the positive fluorescence reaction is provided by the absence of fluorescence in chromosomes to which 5S RNA had been hybridised but which were treated with antiserum absorbed with poly(rA)·poly(dT) (5 μg poly(rA)·poly(dT) per μl serum for 24 h at 4 °C, centrifuged for 10 min at 6,000*g*). Thus the immunological reagents revealed only DNA–RNA hybrids within the nuclei of polytene cells prepared for *in situ* hybridisation.

The specificity was demonstrated further by analyses of the immunological properties of the antiserum. As described previously, several-thousand-fold dilutions of serum reacted in complement fixation assays with poly(rA)·poly(dT), poly(I)·poly(dC) or hybrids of natural RNA and DNA (ref. 1). A 1/50 serum dilution did not react with any single-stranded form of RNA or DNA or with double-stranded RNA or native DNA. When serum was assayed undiluted in counterimmunoelectrophoresis, weak reactions were seen with poly(rA) and with denatured DNA. Both these reactions were eliminated when the serum was passed through a poly(rA)–Sepharose affinity column prepared as described by Poonian *et al.*[10] (Fig. 3). With absorbed serum, which gave the same immunofluorescence as unabsorbed serum, the hybrid was the only reactive polynucleotide class, even in assays with undiluted serum.

There is a variable amount of fluorescence in cytoplasmic components, the origin of which is not yet known. Experiments are projected to attempt to block it while leaving the activity against hybrid nucleic acids intact. Occasional pale fluorescence observed in nucleoli is attributed to contamination of the 5S rRNA probe with fragments of 18S and 28S nucleolar rRNA,

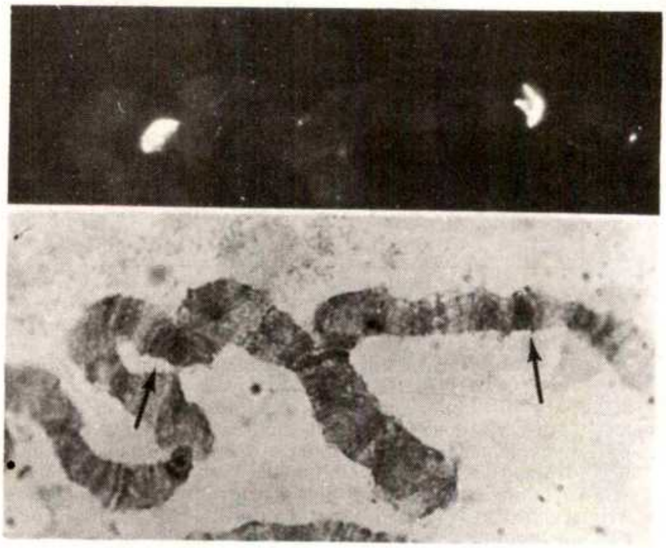

Fig. 1 5S rRNA genes revealed in polytene chromosome 2R of *Drosophila melanogaster* by indirect immunofluorescence detection of RNA–DNA hybrids formed *in situ*. The two homologous 2R chromosomes are not paired except in their most distal portions. The 5S genes (56F on Bridges' standard map) are in the unpaired portions, those derived from one parent to the right, the other parent to the left. In each homologue at least two fluorescent cross-bands are visible. Arrows indicate the 56F regions in the upper photograph of the same chromosomes taken after staining with aceto-orcein. A salivary gland from a fully grown larva of *D. melanogaster* (giant phenotype) was fixed in 50% (v/v) aqueous acetic acid and squashed under a siliconed cover glass, then the slide was frozen on solid CO_2. After snapping off the cover slip, the slide was post-fixed in 3 : 1 ethanol–acetic acid (v/v), rinsed twice in 95% aqueous ethanol (v/v) and stored in 95% ethanol until used. Subsequent treatments were carried out in a moist chamber consisting of a 90-mm square culture dish containing a few leaves of bibulous (or filter) paper saturated with the solvent and two plastic strips to raise the slides above the wet paper. The reagent was placed between the slide and a cover slip. For hybridisation *in situ*, slides were first treated with pancreatic ribonuclease, 100 μg ml^{-1} of 2 × SSC (SSC is 0.15 M NaCl and 0.015 M sodium citrate) for 2 h at 25 °C, then with 90% formamide in 0.1 × SSC for 2 h at 65 °C followed by ice cold 0.1 × SSC rinses. The hybridisation reaction was carried out for 22–24 h at 37 °C in 50% formamide in 4 × SSC using either 1.0 or 0.2 μg 5S RNA per slide—highly purified 5S rRNA, extracted from *D. melanogaster* Oregon R embryos[11]. After the annealing reaction, the slides were treated with pancreatic ribonuclease (15 μg ml^{-1}) in 2 × SSC for 2 h at 25 °C, rinsed with phosphate-buffered saline (PBS, 0.14 M NaCl, 0.01 M phosphate, *p*H 7.2) and exposed for 2 h at room temperature (21–23 °C) to rabbit anti-DNA–RNA hybrid serum reconstituted from a lyophilised state by solution in water and diluted for use in PBS (1 : 20 for Fig. 1). After thorough rinsing in PBS, the slides were finally exposed to a rhodamine-labelled goat IgG fraction of anti-rabbit-IgG (Miles-Yeda) reconstituted to approximately its original concentration, then diluted in PBS for use (1 : 20 for Fig. 1). The photographs were taken with a microscope equipped with a Zeiss epi-illumination fluorescence module using a 546-nm excitation filter, a 580-nm chromatic splitter and a 580-nm barrier filter for rhodamine fluorescence on 35-mm Eastman Tri X Pan film exposed at ASA 1600 (Diafine developer) at magnifications of approximately × 150 (× 40 objective) or × 370 (× 100 oil immersion objective). Fluorescence exposures were in the range of 1–8 s and phase contrast illumination was adjusted to require approximately the same exposure time so that a single frame could be exposed in both modes simultaneously (not shown here). In some instances, slides were stained with aceto-orcein after fluorescence photography had been completed, then photographed through a No. 58 filter (green) on Plus-X-Pan film at ASA 400. Approx. × 1,000.

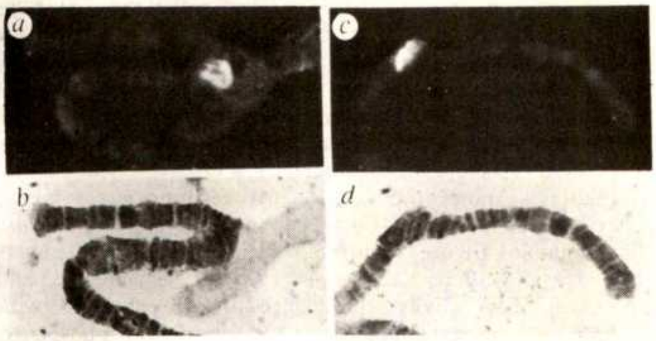

Fig. 2 5S rRNA genes revealed in *D. melanogaster* polytene chromosome 2R as in the legend to Fig. 1. The two chromosomes are unpaired in a single nucleus. The upper chromosome (*a* and *b*) is associated with the nucleolus (nu) at the locus of the 5S genes, fluorescent in (*a*). The homologous chromosome (*c* and *d*) is not visibly associated with the nucleolus; its 5S locus, indicated by the fluorescence in (*c*), is less disperse than that in its homologue (*a*). (*b* and *d*) Photomicrographs of the same fields as in (*a*) and (*c*), respectively, after staining with aceto-orcein. × 630.

but the possibility that 5S DNA templates occur in nucleoli cannot be excluded.

The spatial resolving power of the immunofluorescent probe is equal to that of the optical system used to observe it. Autoradiographs can at best reveal a cluster of silver grains adjacent to, or covering, a labelled region which results in a resolving power of the order of 1–3 µm. So far the precise localisation of the 5S genes within the 56EF region has been equivocal for *D. melanogaster* using autoradiography[1-9]. The images we obtain are of two kinds. In some nuclei, the fluorescence is restricted to a relatively narrow transverse 'band' which is often clearly made up of two subunits and is localised in the 56F region, distal to the puff usually present in 56E (Fig. 1). The possibility that the two subunits reflect the organisation of the 5S locus into the two separable sets of repeated sequences recently reported by Procunier and Tartof[12] is under investigation. On the other hand, the 5S region of chromosome 2R sometimes sticks to the nucleolus as in Fig. 2 (ref. 9) or is ectopically paired to other chromosome regions. In those cases, the fluorescence may be distributed in a network of fibrils extending longitudinally along a much longer segment of chromosome (Fig. 2*a*). When that is true, the morphology in phase contrast and/or after post-staining with aceto-orcein is atypical in that the subsections 56E and F cannot be clearly demarcated and the region does not appear to be organised into distinct bands. Such dispersion of the *in situ* hybrids into fibrils is consistent with the suggestion of Steffensen and Wimber[9] that the 5S genes may have been active in those chromosomes[13]. But the possibility that our 5S probe contains traces of contaminating nucleolar rRNA fragments that could reveal nucleolar rDNA adhering to chromosome 2 has not been entirely excluded.

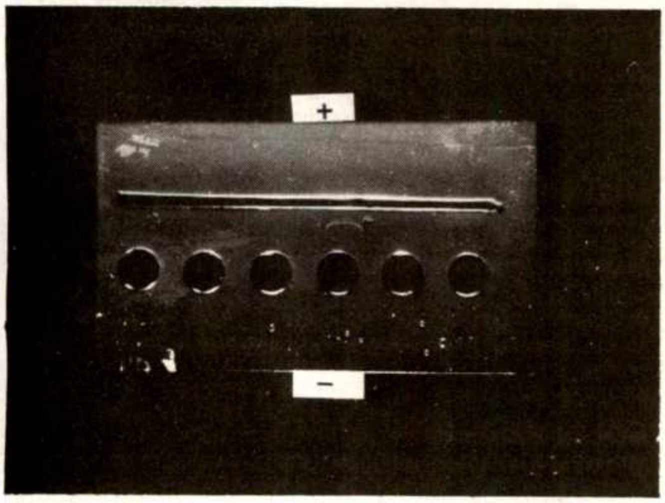

Fig. 3 Specificity of the absorbed antiserum. A sample of 7 ml of anti-poly(rA)·poly(dT) antiserum was absorbed by passage through a 1.2-ml column of poly(rA)–Sepharose equilibrated with 0.1 M NaCl, 0.01 M phosphate, *p*H 7.2. The undiluted absorbed serum (200 µl) was placed in the trough; the wells contained 0.5 µg polynucleotide in 50 µl running buffer (0.05 M Tris-HCl, *p*H 8). The polynucleotides were, from left to right: poly(rA); denatured DNA; poly(rA)·poly(dT); poly(dT); poly(I)·poly(C); and poly(dA). The gel medium was 0.8% agar (Difco, purified agar) in running buffer; 7 ml of gel was poured on each 2 × 3 inch glass plate. Electrophoresis was run at 220 V (10 m per plate) for 45 min.

The sensitivity of the technique has not yet been fully explored. At serum dilutions of 1 : 40 for the anti-hybrid rabbit serum and an equivalent concentration for the fluorescent reagent, the fluorescence intensity in the 5S region was very high. Photographic images should still be easily recordable at brightnesses one to two orders of magnitude lower. Thus, the possibility to detect the hybridisation of RNA copies of a unique gene in a polytene chromosome appears to be real. On the other hand, genes present in a size and multiplicity equivalent to the 5S of *D. melanogaster* may be detectable in uninemic chromosomes. An attempt is in progress to detect the 5S locus in human chromosomes at the pachytene stage of meiosis.

The technique is being used to study the distribution of naturally occurring chromosomal RNA detected as hybrid molecules. Polytene chromosomes mounted out of 50% acetic acid display a pattern of fluorescent regions when treated only with the immunological reagents. The regions do not fluoresce if the anti-hybrid serum is blocked with poly(rA)·poly(dT) or if the chromosomes are treated to remove indigenous chromosomal RNA (as in preparation for *in situ* hybridisation), indicating that they are sites of hybrid molecules. Since the locations of the sites change during larval and prepupal development, their RNA moiety could be involved with the control of transcription or of replication or both.

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Anion modulation of the negative Bohr effect of haemoglobin from a primitive amphibian

THE Congo eel (*Amphiuma means*) is a primitive salamander that respires through its lungs, gills and integument. It lives in swamps, ditches and rice fields in the south-eastern USA. When pools and swamps become stagnant and water levels drop, *Amphiuma* will burrow and hibernate in the mud. In this letter we report that when organic phosphate cofactors are low or absent, as might be true during hibernation, the haemoglobin of this amphibian has unusual properties which may help the animal to extract oxygen from a low *p*H environment. The molecular adaptation exhibited by *Amphiuma* haemoglobin is a *p*H dependence of oxygen binding that is reversed relative to that of most haemoglobins. Low *p*H brings about a large increase in the oxygen affinity of stripped *Amphiuma* haemoglobin. This negative Bohr effect indicates that protons are bound as oxygen is bound. This is in contrast to the release of protons that usually accompanies oxygenation. New Bohr groups must be present in this amphibian haemoglobin, or the normal Bohr groups must be drastically modified. From a physiological viewpoint it is of interest that the Bohr effect *in vitro* is subject to regulation by organic phosphate cofactors.

Haemolysates from the two *Amphiuma* studied gave single somewhat broadened bands on polyacrylamide disc gels, indicative of a single major haemoglobin. There were no minor components, even on overloaded gels. Haemoglobin solutions were prepared by the ammonium sulphate procedure and stripped of organic and inorganic ions by treatment with a mixed-bed ion exchange resin[1]. Oxygen equilibria were done spectrophotometrically using a tonometric procedure[2], and flash photolysis and rapid mixing experiments were done as described before[3].

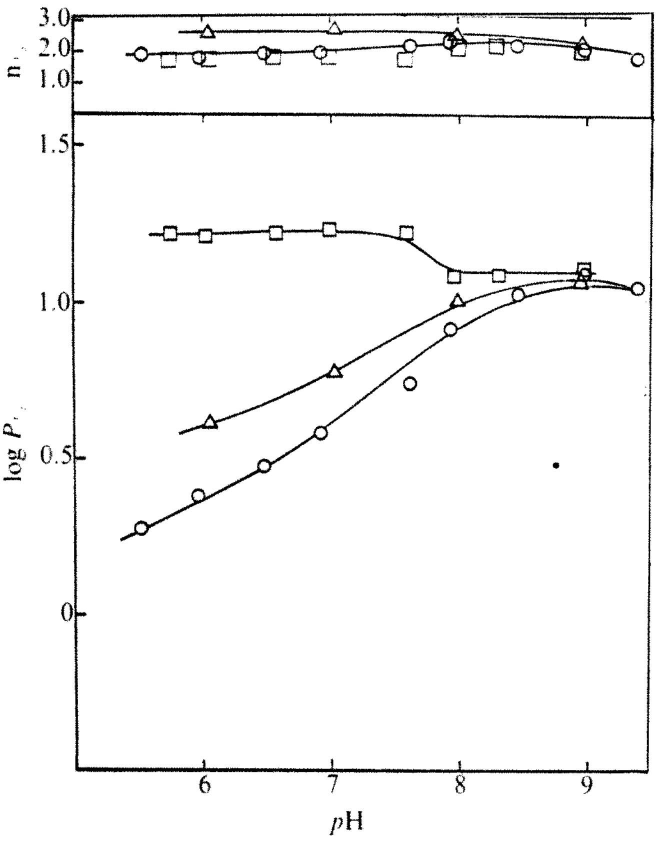

Fig. 1 Effect of *p*H and cofactors on the oxygen affinity and cooperativity of *Amphiuma* haemoglobin (~60 μM in haem) in 0.05 M bis-Tris or Tris buffers at 20 °C. Solutions of stripped haemoglobin were examined in the absence of cofactors (○), with 0.3 M NaCl (△), and with 100-fold excess of ATP:tetramer (□). Oxygen pressures measured in mmHg.

Figure 1 shows the large negative Bohr effect which characterises oxygen binding to stripped *Amphiuma* haemoglobin. Figure 1 also shows that 0.3 M NaCl lowers the oxygen affinity, decreases the *p*H dependence and increases cooperativity. Similar results are obtained with *Amphiuma* haemoglobin in 0.2 M inorganic phosphate. Organic phosphates cause an even greater decrease in oxygen affinity, although they do not appreciably alter the degree of cooperativity. Table 1 summarises these results and presents data on human haemoglobin A (HbA) for comparison. There are significant quantitative differences between HbA and *Amphiuma* haemoglobin in the relative effectiveness of various organic phosphates.

Table 1 Effects of anions and *p*H on the oxygen affinities of stripped preparations of *Amphiuma* hemoglobin at 20 °C in 0.05 M bis-Tris or Tris buffers (except for experiments in 0.2 M phosphate)

Haemoglobin	Cofactor	$P_{\frac{1}{2}}$(mmHg) *p*H 7	$P_{\frac{1}{2}}$(mmHg) *p*H 8	Bohr effect $\Delta\log P_{\frac{1}{2}}$ (*p*H 7–8)
Amphiuma	none	4.0	8.3	−0.32
	0.3 M NaCl	5.9	10.2	−0.24
	0.2 M phosphate	5.1	8.9	−0.24
	2,3-diphosphoglycerate	6.7	—	—
	adenosine triphosphate	17.4	12.3	+0.15
	inositol hexaphosphate	20.0	13.5	+0.17
HbA	none	2.0	0.5	+0.56
	1.0 M NaCl	7.9	3.1	+0.41
	0.2 M phosphate	12.6	3.5	+0.55
	2,3-diphosphoglycerate	12.6	3.1	+0.60
	adenosine triphosphate	10.0	2.2	+0.66
	inositol hexaphosphate	38.9	11.5	+0.56

Data on human haemoglobin (HbA) are presented for comparison.

Enzymatically modified haemoglobin has been used to probe the mechanism of ligand binding. The properties of HbA after digestion with carboxypeptidase A and B, in particular, helped establish the important role played by its carboxyl-terminal residues[4–6]. Properties of carboxypeptidase A and B digests of *Amphiuma* haemoglobin, prepared as described elsewhere[7], are shown in Fig. 2 and Table 2. The functional properties of the digestion products differ from those of carboxypeptidase digests of HbA. Interpretation of these differences is difficult because there are no structural data available on *Amphiuma* haemoglobin. We presume it to be an $\alpha_2\beta_2$ tetramer largely by analogy with other amphibian haemoglobins, and in part because of the identity of the amino acids released as the result of carboxypeptidase digestion[8,9]. Carboxypeptidase B digestion of HbA removes arginine from its alpha chains. Similarly, arginine is the only residue released from a comparable digest of *Amphiuma* haemoglobin. Digestion with carboxypeptidase A causes β146 histidine and β145 tyrosine to be cleaved from the C-terminus of the beta chain of HbA. A comparable digest of *Amphiuma* haemoglobin releases only tyrosine.

The β146 histidine of HbA is thought to be responsible for much of the positive Bohr effect of HbA[4,5]. Consequently, absence of C-terminal histidine in *Amphiuma* haemoglobin may help explain its altered *p*H dependence. It is interesting that stripped tadpole haemoglobins show negative Bohr effects even though they possess a C-terminal histidine[9]. Amphibian haemoglobins typically have low Bohr effects[8], and absence of the C-terminal histidine in *Amphiuma* haemoglobin may therefore only accentuate a common divergence from mammalian haemoglobins.

The β145 tyrosine of HbA is thought to play a major role in stabilisation of the low affinity (T) conformation[4]. Removal of this residue is responsible for the high affinity and lack of cooperativity exhibited by HbA after digestion with carboxypeptidase (refs 4–6). A Carboxypeptidase A digestion of *Amphiuma* haemoglobin does not eliminate cooperativity or greatly increase its oxygen affinity, which suggests that the C-terminal tyrosine of this haemoglobin is not as important in stabilisation of its low affinity state. Sensitivity to organic phosphates is maintained by the carboxypeptidase A digest and the Bohr effect is rendered positive by the addition of adenosine triphosphate or inositol hexaphosphate.

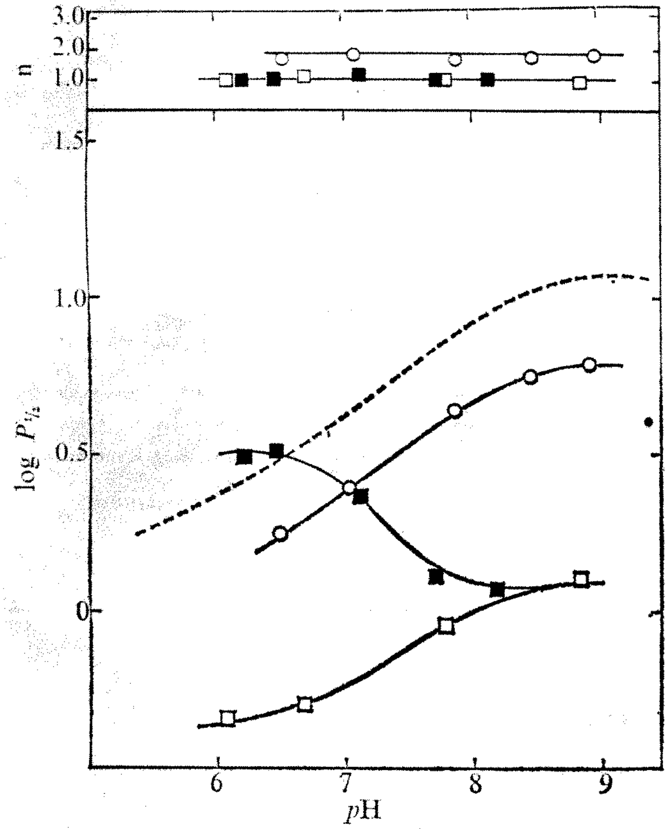

Fig. 2 Effect of *p*H on the oxygen affinities of carboxypeptidase A (○) and carboxypeptidase B (□) digests of *Amphiuma* haemoglobin. The effect of inositol hexaphosphate (■) on the CPB-digested material is also shown. Stripped *Amphiuma* haemoglobin – – –. Other conditions as in Fig. 1.

The β141 arginine of HbA is thought to contribute to stabilisation of the low affinity (T) conformation by formation of ligand-linked salt bridges[4–6]. Loss of this residue after carboxypeptidase B digestion of HbA causes a moderate increase in affinity and a decrease in cooperativity. In *Amphiuma* haemoglobin the C-terminal arginine is much more important, as demonstrated by the greatly increased oxygen affinity and loss of cooperativity which accompany digestion with carboxypeptidase B. It is remarkable that this non-cooperative digestion product has a strong negative Bohr effect. In the presence of inositol hexaphosphate, however, the carboxypeptidase B digest of *Amphiuma* haemoglobin shows a positive Bohr effect, comparable to that of HbA.

The kinetics of ligand binding by *Amphiuma* haemoglobin differ greatly from those of most other haemoglobins in that the typical *p*H sensitivity is reversed. For most haemoglobins the rate of oxygen dissociation is much faster at *p*H 7 than at *p*H 9, while the rate of ligand combination is slower at *p*H 7 than at *p*H 9. These kinetic factors work together in generating the positive Bohr effect of most haemoglobins. In contrast, Fig. 3 shows that low *p*H favours a slow rate of oxygen dissociation and a fast rate of CO combination for stripped *Amphiuma* haemoglobin. The *p*H dependence of these processes adequately accounts for the negative Bohr effect (Fig. 3). In accord with the equilibrium data, adenosine triphosphate (Fig. 3) alters these kinetic processes at neutral *p*H, with little effect at high *p*H.

At neutral *p*H the time course of CO binding to a concentrated solution of HbA shows an increasing rate as successive ligands are bound, indicative of cooperative subunit interactions[1]. Similarly, the apparent rate constants increase 1.5–2-fold during the process of CO binding to *Amphiuma* haemoglobin. Flash photolysis of *Amphiuma* haemoglobin at low heme concentration (~ 0.5 μM) gives no indication of quickly reacting material, and the autocatalytic character of the time course is maintained. This suggests that liganded *Amphiuma* haemoglobin does not readily dissociate into non-cooperative subunits. It will be of considerable interest to determine if *p*H or cofactors affect the state of aggregation of the protein. The time course of O_2 dissociation from stripped *Amphiuma* haemoglobin was also somewhat autocatalytic, although this was not as pronounced as in the kinetics of CO combination.

The foregoing serves to illustrate the unusual *p*H sensitivity of *Amphiuma* haemoglobin. Bohr effects that are low or slightly negative have been reported for haemoglobins from a number of other amphibians[8], and we suspect that this characteristic may be a molecular adaptation to meet respiratory requirements during hibernation. None of the amphibian haemoglobins so far investigated has a negative Bohr effect as pronounced as that of *Amphiuma* haemoglobin, and no other cases of reversed *p*H sensitivity of both CO combination kinetics and O_2 dissociation kinetics have been reported.

The flexibility of haemoglobin function is brought into focus by this study by the fact that homotropic and heterotropic interactions can vary independently of one another. This is significant because homotropic and heterotropic interactions are tightly linked in normal human haemoglobin. Our functional studies with *Amphiuma* haemoglobin illustrate that the Bohr effect can be positive or negative without appreciable alteration of cooperative

Table 2 Effects of anions and *p*H on the oxygen affinities and cooperativity of stripped preparations of chemically modified forms of *Amphiuma* haemoglobin. Data collected at 20 °C in 0.05 M bis-Tris or Tris buffers

Modification	Cofactor	$P_{1/2}$ (mmHg) *p*H 7	$P_{1/2}$ (mmHg) *p*H 8	$n_{1/2}$
Carboxypeptidase A digest (des Tyr)	None	2.5	4.42	1.7
	Adenosine triphosphate	7.4	—	1.5
Carboxypeptidase B digest (des Arg)	None	0.6	1.0	1.0
	Adenosine triphosphate	1.7	1.0	1.0
	Inositol hexaphosphate	2.6	1.2	1.0

interactions and that these changes in *p*H sensitivity can occur even in the absence of cooperative oxygen binding. In *Amphiuma* haemoglobin the separation of heterotropic and homotropic interactions is much more evident than in most other haemoglobins. Further study along these lines should contribute to our understanding of the flexibility of haemoglobin function in response to environmental pressures.

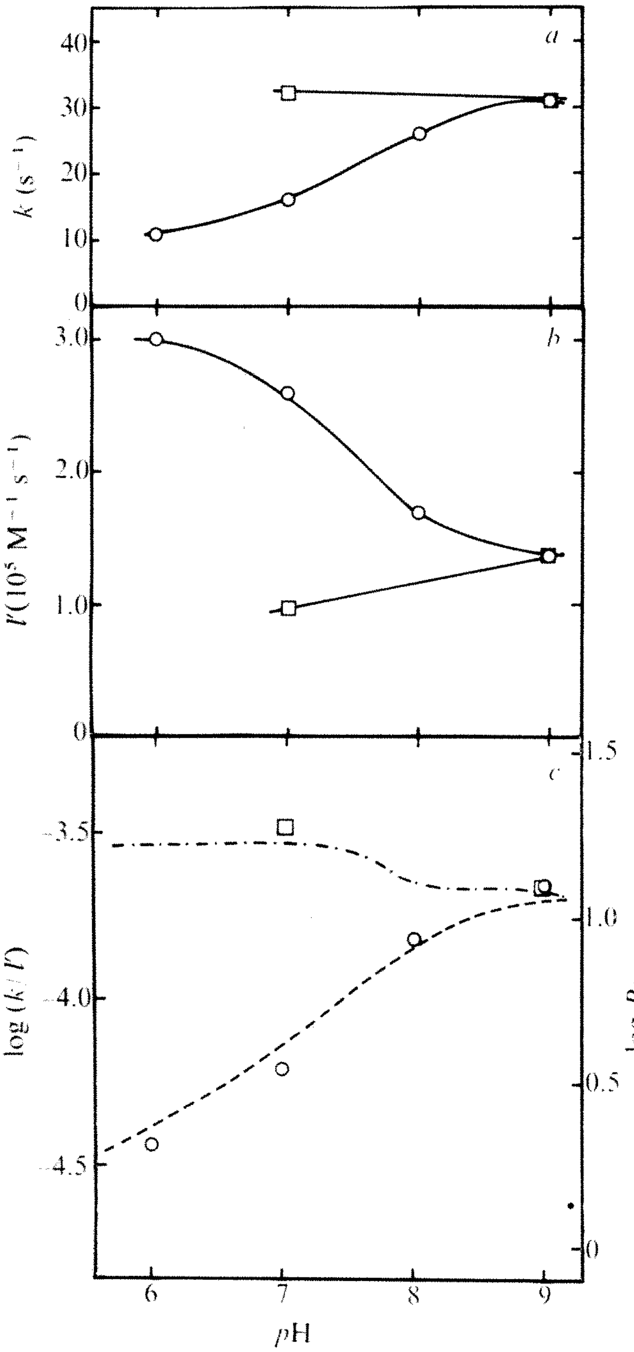

Fig. 3 Effect of *p*H on the apparent velocity constants at 20 C for oxygen dissociation (*a*) and CO combination (*b*) for stripped *Amphiuma* haemoglobin (○) and for the haemoglobin in the presence of 1 mM ATP (□). *c*, The log of the ratio of the kinetic constants (symbols) is compared to the equilibrium data of Fig. 1 (dashed lines).

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Errata

In 'Release of substance P from isolated nerve endings' by Christina Schenker, Edmund A. Mroz and Susan E. Leeman (*Nature*, **264**, 791, 1976), line 9 should read . . . not be released by high K$^+$ in the presence of Ca^{2+} . . .

In 'Significant journals of science' by E. Garfield (*Nature*, **264**, 609–15), on page 610, in Fig. 1*a*, the headings of the three columns to the right of the column of journal titles should read D, E and F. On page 612, in Fig. 2*a*, the column headings A, B and C should appear above the three columns on the left. On page 614, in the legend for Fig. 3, the definition of column D shown read "number of 1974 articles".

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reviews

Hard reason and deep humanity

J. W. S. Pringle

Insects and the Life of Man: Collected Essays on Pure Science and Applied Biology. By Sir Vincent Wigglesworth. Pp. 217. (London: Chapman and Hall, 1976. Distributed in the USA by Halsted Press, a Division of Wiley: New York.) Paperback £3.25; hardback £6.

THIS book is a collection of essays or addresses published over a period of nearly forty years. Many of them are concerned with aspects of applied entomology and its relation to 'pure' science, but the life of man means more than just his material needs. So it is appropriate that the volume includes some of the contributions which Professor Wigglesworth has made to the history and philosophy of science and, in the widest sense, to its art.

The introductory chapter gives a guide to what follows. The earliest contribution is an article published in 1935 on malaria in Ceylon, which may well have stimuated the successful WHO campaign after the war. Wigglesworth did not foresee the problems that would arise from the resulting population explosion, but he did clearly forecast in 1945 the ecological effects that would result from widespread use of DDT. By 1951 he was acknowledging that "there can be no immediate prospect of abandoning the use of insecticides" but was pleading for more knowledge, with which "we should be able to devise methods of cultivation which would favour the crop and discourage the insect pest to the point where a satisfactory balance is established". This was well before the invention of "integrated control".

The essays are not primarily autobiographical, but chapter 12—an address to XIIth International Congress of Entomology in 1964—does start with a brief summary of Wigglesworth's career. Few people know that the *Rhodnius* cultures now so widespread in biological laboratories derive from a small stock imported from Venezuela about 1924 and laboratory-bred ever since. There follows a review of fifty years' progress in many fields of insect physiology since 1912—"impressive examples of the potential contribution of insect physiology to general biology". Yet, "I believe that the great era of insect physiology is yet to come": with the fertile observation that metamorphosis is a genetic switch, a step-wise activation of particular components of the genetic code, and that because in insects the switch is controlled by a single chemical, the juvenile hormone, "there is no more promising medium than the insect, in which this problem can hopefully be studied".

There is a stimulating essay (given to the British Association in 1948) on the history of entomological studies in Britain and the relationship between applied entomology and research. I particularly like the remarks about administration: "The keynote [of administration] should be the safeguarding and encouragement of research. Anyone who has tried both knows that administration is so immeasurably easier than research that it becomes the line of least resistance, and that is why research needs encouragement". Entirely historical is a sketch of the contributions of Sir John Lubbock (Lord Avebury) to insect physiology, "to put the record straight". "If Lubbock had held an academic appointment, he would have been accorded a high place among the biologists of the nineteenth century. But, like Darwin, he was an amateur; and unlike Darwin, he devoted only a minute fraction of his time to scientific pursuits".

Chapter 13 reprints a remarkable article on the epidermal cell, which is often overlooked since it was published in a *Festschrift* after delivery as a lecture to the Royal Society. The diversity of functions which these cells can perform is one of the most remarkable phenomena in the whole of biology. There is material here for a generation of PhD theses.

The man behind the words comes through most clearly in chapter 16 (Wordsworth and Science) and in chapters 4 and 17 (Science, Pure and Applied; The Religion of Science). He admires Wordsworth "who, standing at the threshold of the Industrial Revolution . . . can compare with true scientific objectivity, though with all a poet's feeling, the evil of the factory child with the grinding penury of rural England" and who can, in 1810, find a solution and a hope in "a System of National Education established universally by Government". In chapters 4 and 17, Wigglesworth sets out clearly his faith, on which science is founded and by which it lives. "Without a deeply rooted instinctive belief in the existence of laws which reign throughout nature, the incredible labours of scientists would be without hope". Some rude things are said about philosophers, but the lecture was "anti-philosophical only to the extent that it takes the view that science and idealist philosophy have different ground rules and therefore cannot play together". The picture is of a man of hard reason, coupled with deep humanity.

I have only sampled some of the gems in this book. □

J. W. S. Pringle is Linacre Professor of Zoology at the University of Oxford, UK.

Epidemics and pestilences

Insects and History. (The World Naturalist Series.) By J. L. Cloudsley-Thompson. Pp. x+242. (Weidenfeld and Nicolson: London, 1976.) £8.50.

THIS is a history book about pestilences, from the dawn of history to the present day. It is vividly written and well documented. Much of it makes horrifying reading. As the story progresses it seems to support the popular view that great epidemics are a thing of the past. But that past is not so very distant: the influenza pandemic of 1918 is thought to have resulted in 20 million deaths—a larger number than any other pestilence in history. The chapter headings read like an account of medical entomology. But the value and interest of the book is enhanced by the large amount of space given to smallpox, scurvy, syphilis, famine and, of course, to the mixed infections and unidentified diseases which defy diagnosis at this distance in time.

The author is cautious in his attributions of historic change to specific insect-borne diseases. The well known idea of W. H. S. Jones that the loss of vigour among the Greeks when the power of Rome was increasing, was the result of malaria. But the vigour of a people can be sapped from other causes; and when malaria does increase it is not always easy to distinguish cause and effect. Did the collapse of the Sinhalese civilisation in Ceylon result from the introduction of malaria? Or did malaria increase because the repeated Tamil invasions from the mainland disrupted the elaborate system of irrigated agriculture established by the Sinhalese. After all, it was probably improved and intensive agriculture which dispelled malaria from East Anglia in the last century. There seems little doubt that malaria in the Pontine marshes was a real factor among the epidemics which destroyed successive armies invading Rome. But in the inverse relationship pointed out by Celli between agricultural productivity in the Roman Campagna and the incidence of malaria, it may well have been intensive agriculture which was controlling malaria in ancient times as well as in the time of Mussolini.

It is a popular idea that malaria and swamps go hand in hand, and nothing is said in this book to dispel this belief. It happens to be true for much of Europe; but it is the reverse of the truth in most of the tropics, where the Anopheline mosquitoes which infest swamps and paddy fields are usually not carriers of malaria at all. The roadside puddle and the grassy streamlet are infiinitely more dangerous. It came as a surprise to all sides in World War I to discover that in the Mediterranean area the mountain streams were often the major source of malaria.

This is a book about epidemics and pestilences, it is not about medical entomology. The insect carriage of disease is a subtle matter. Even the subtleties that are known scarcely appear in this book; and there is much that is not known. The author makes no pretence that the epidemiology of infectious diseases is a simple matter: he could hardly have covered the complexities of insect transmission as well as the rich history of epidemics that he provides. When the International Health Board of the Rockefeller Foundation was set up, it decided to make a big splash by tackling a project which it knew would be successful. General Gorgas had saved the labour force of the Panama Canal from yellow fever by first-class organisation of simple procedures. The International Health Board would banish yellow fever from the New World. That was in about 1912. In 1917 they claimed complete success, but a year or so later a small outbreak occurred and they had to do it again. And so it went on for years, until it was discovered by Soper in 1935 that the yellow fever virus was being maintained in the Amazon jungle by forest mosquitoes feeding on monkeys in the treetops. When small clearings were made men were infected and returned to the towns, where they started epidemics carried by the urban mosquito *Aedes aegypti*. The Rockefeller Foundation had been unlucky; they had chosen a disease the eradication of which was impossible.

The author is an assiduous quoter of chapter and verse. There are times when the reader becomes tired of the repetitive statistics of horror. The effect, however, is cumulative, and the docketing of the fate of successive naval expeditions from the sixteenth century onwards, when faced with malaria, yellow fever, scurvy and the rest, bring home the facts in a way that no general account could do. The same applies to the figures for the incidence of typhus deaths in the wars of the eighteenth and nineteenth centuries. We all know that until recently the major cause of death in war was epidemic disease. But when the figures are set out as here in detail, they are staggering.

In a concluding chapter the author deals with insects harmful to agriculture, concentrating his attention on the plague locusts which, as a major cause of famine, contribute greatly to the incidence of epidemic disease in man. He sketches also the former role of insects in commerce—as a source of dyes, waxes and lac, honey (for long the main source of sweetness) and silk, which played such an important part in the world trade of ancient China. All these products have receded in importance in modern times, but their impact on economic history as here recorded is fascinating.

Altogether this is a book that is full of intriguing information which cannot fail to interest both the general reader and the informed entomologist.

V. B. Wigglesworth

Sir Vincent Wigglesworth is Emeritus Professor in the Department of Zoology, University of Cambridge, UK.

Man's ectoparasites

Insects, Hygiene and History. By J. R. Busvine. Pp. 262. (Athlone: London, 1976. Distributed by Tiptree Book Services: Tiptree, Essex, and Humanities: New Jersey.) £6.95.

MOST of us who have lectured in medical entomology have tried to enliven our discussion of human ectoparasites by including a few anecdotes such as that demonstrating the holiness of St Thomas of Canterbury, whose garments, after his murder in the cathedral, "boiled over with lice like water simmering in a cauldron". This was, of course, in an age when cleanliness was considered to be the antithesis of godliness. We realised that there was a wealth of entertaining and instructive fact and fiction buried in the literature from the times of classical antiquity to the present day. A few writers—for instance, Hans Zinsser in his famous book *Rats, Lice and History* —have hinted at the richness of the information waiting to be revealed by some student of the byways of parasitology, but others, up till now, have not had the patience to follow his lead.

At last Professor J. R. Busvine has accepted this implicit challenge, and has written the first comprehensive account of the history, and the mythology, of man's ectoparasites. He has produced a most entertaining text, full of tales of observation and of misunderstanding, of ribald verse and donnish pornography. Future lecturers will have no excuse if they make parasitology a dull topic.

Hair hygiene to eradicate lice

Hortus Sanitatis (1491)

This book has clearly been compiled during a lifetime study of insects and disease. James Busvine is not primarily a historian although he has done his historical research thoroughly and competently. He has systematically noted down each reference he has come across, sometimes by chance, often only after considerable searching, during the course of his major studies. But the great value of the book is that its author has himself contributed substantially to modern knowledge of its subject, so that he relates the historical observations, with all their curiosity, to the situation as understood by modern science.

This book is, however, more than an entertaining account of man's frailties. It shows how science progresses, and how this progress is often delayed. It is

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often surprising to find that the same person could make accurate observations and draw the correct conclusions from them, and at the same time seem to be quite blind in another, though similar, field. Thus Aristotle was in some ways the father of scientific zoology, and many of his descriptions of animals and plants need little modification today. Yet, although he described the nit (the egg of the louse), he was convinced that it had no generative function, and that these insects arose only from the putrefaction of human flesh. This view was held for centuries, and to deny it could lead to a charge of heresy by the mediaeval church. Before the invention of the microscope naturalists could be excused for many misunderstandings, but it is difficult to comprehend why they were so badly mislead regarding this parasite, which can be so easily studied throughout its life history with the naked eye.

But the situation may not have changed as much as we may like to think. Scientists and clinicians still find it difficult to accept new evidence and relinquish long-held beliefs. I well remember how incredulous some dermatologists were when, during the last war, we demonstrated that the itch mite, the cause of the disease of scabies, was usually transmitted by close personal contact and seldom by blankets and other inanimate objects. One leading physician stated publicly that these scientific experiments were all very well, but he *knew*, from a lifetime of clinical experience, that blankets and other fomites were of major importance. Fortunately he could not call on the inquisition to stifle our results, which were soon accepted by his colleagues and by the Ministry of Health, so that treatment was improved and the disease was, temporarily, nearly erradicated in Britain.

Kenneth Mellanby

Kenneth Mellanby was Reader in Medical Entomology in the University of London (1945–47) and Professor of Parasitology in the University of Ibadan (1948–53).

How behaviour works

The Hungry Fly: A Physiological Study of the Behaviour Associated with Feeding. By V. G. Dethier. Pp. 489. (Harvard University: Cambridge, Massachusetts, 1976.) £20.40.

THE appetites for sex and food are the two most important driving forces in animal behaviour, the one to maintain the species and the other to enable the performance of the first. Research on these appetites is therefore central to the study of behaviour. Most of our knowledge of the mechanisms involved in the appetite for food derives from work on the rat—an animal whose mentality is perhaps dangerously too near our own for complete objectivity. Some 30 years ago Dethier chose to work on the hunger of a simpler organism, the blowfly, with whom he and his students were less likely to identify. The result has been a volume of research scarcely less impressive than that on the rat, and in some respects much better in quality, notably on the electrophysiological correlates of taste and feeding.

The Hungry Fly is a kind of autobiographical account of these 30 years of research. It is difficult to categorise precisely, since the author seems not quite to have made up his mind whether to write a popular text or an advanced treatise. Thus, each chapter opens with a few rather elementary pages, some sprinkled with statements that will seem either banal or erroneous to the professional biologist; but then plunges into the technicalities of chemosensory transduction or perseverating central excitatory states. The professional may be irritated by the introductions, the lay reader surely rapidly lost by the rest.

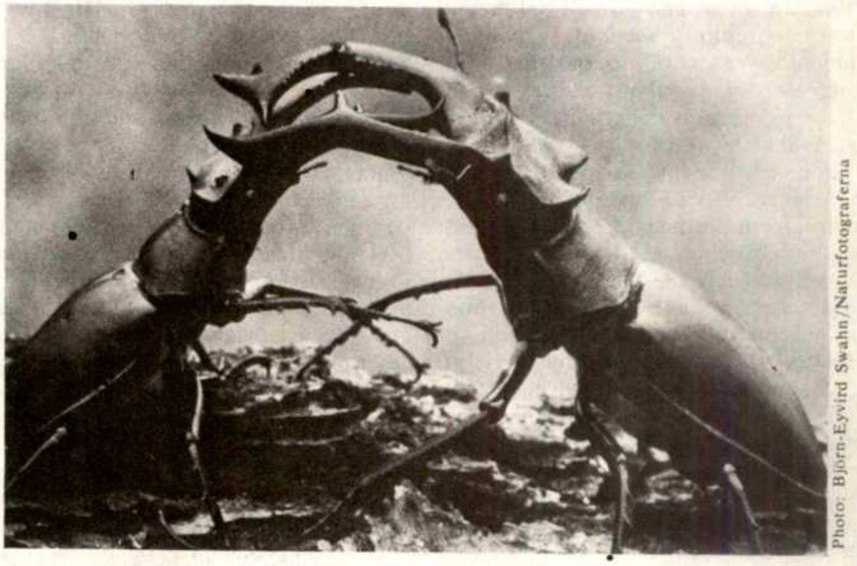
As one reads through the minutiae of experiments carried out in the 1950s that have since been superseded, one does begin to wish that the account was slightly less full. The book could surely have been slimmer, cheaper, and more assimilable if these false trails and some of the other fine detail had been omitted. Chapter 12, for example, describes at great length the neuroanatomy of the fly's brain with the aid of over 40 light micrograph serial sections (incidentally, without indicating scale, section position or thickness) and then ends frustratingly by saying that nothing whatever is known about the functional organisation of the dipterous brain.

Dethier uses the Lorenzian concepts of 'appetitive behaviour' and 'consummatory act' throughout his book, but the reader should not be thereby misled into thinking that he is still of that school of behaviourists who believe in these psychological notions of drive and motivation. His last chapter, perhaps the best in the book, spells out in brief, eloquent simplicity why there is no need to invoke these anthropomorphisms to explain the workings of behaviour, and why there is no need to consider the behaviour of the rat as qualitatively different from the simpler behaviour of the insect.

In effect, the book is a three-fold filling out and up-dating of Dethier's long 1969 review on the same subject. He says it is not about chemoreception and neurophysiology (though much of it is), but is a description of behaviour in terms of physiological mechanisms. In this respect it is a superb account, and will surely remain a classic on the shelves of behaviourist-physiologists for many years to come. It will probably also provide the more general biologist with insights into how behaviour works and what it is made of. Whether a wider public will be very much enlightened by it is less clear.

John Brady

John Brady is Reader in Insect Behaviour in the Department of Zoology and Applied Entomology at Imperial College, London, UK.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Fighting stag beetles. Taken from *The Secret Life of Insects* by P. Passarin d'Entrèves and M. Zunino. Pp. 378. (Orbis: London, November 1976.) £8.95.

Economic geology and tectonics

Metallogeny and Plate Tectonics. Edited by D. F. Strong. Pp. vii+660. (Geological Association of Canada: Department of Earth Sciences, University of Waterloo, Waterloo, Ontario, 1976.)

In some areas, the plate tectonic bandwagon must be approaching its elastic limit as more and more ideas and interpretations are heaped on it, yet surprisingly little attention had previously been paid to corallaries of this model for economic geology and Precambrian tectonics. Major progress in both aspects have now been made with the almost simultaneous publication of the proceedings of two separate NATO conferences—B. F. Windley's *The Early History of the Earth* (Wiley, 1976) and D. F. Strong's *Metallogeny and Plate Tectonics*. The latter comprises 26 papers and six abstracts concerned mostly with either general concepts or specific locations, with the main emphasis on attempts to interpret different metallogenies in terms of the current plate tectonic framework. The standard is inevitably variable, but generally very good. The papers are in English, with both English and French abstracts, and it is a credit to the editor that his work on the 'foreign' contributions is undetectable yet must be there for them to flow as well as they do. Similarly the editor must have worked hard to keep out too many pictures of subduction zone models which, although the dominating figure, do not obtrude.

The production is generally good, the only major flaw being that there is no index. This omission is particularly serious for such a heterogeneous collection of papers which, although grouped into sections, contain numerous cases of partial or even complete discussion of features which belong in other sections. This is further aggravated by the order in which the sections are presented: metal deposits in convergent plate margins, intraplate environments, orogenic belts and ores of Precambrian age. This order presumably reflects the fact that orogenic belts contain both subduction and intraplate ore bodies, and Precambrian deposits may contain all forms and also be characterised by modified plate tectonic models resulting from, for example, the much higher average geothermal gradients of Archaean times. Nevertheless, present day subduction zones, such as the Andes and Japan, which form the majority of the areas discussed under convergent zones, also contain pre-existing ore bodies in the same way as the Canadian cordillera, which are discussed under orogenic belts. Such difficulties in grouping the papers would not have mattered so much if the whole volume was indexed, even in a coarse fashion. Under the circumstances, it would have been better to commence with accretion zone deposits, and then have orogenic belts following convergent zones.

Most of the early proponents of metallogeny and plate tectonics have contributed to this publication, but it is significant that the 'grey-beards', in this subject, such as R. H. Sillitoe, must be considered those who published on the subject in or before 1973, and sufficient credit is still not given to those geochemists who determined the zonations in island arc environments well before the discovery of plates. Those papers by E. Horikoshi (north-eastern Japan), A. Y. Glikson (Archaean to early Proterozoic Shield structures), T. Sato (green tuff province, Japan), F. J. Sawkins (massive sulphides), R. A. Stacey (Canadian cordillera) and H. S. Swinden and D. F. Strong (Appalachians, North American cordillera, south-eastern Australia) are particularly interesting. Others are disappointing rather than unimportant. For example, that by P. Laznicka (lead deposits on global plate model) could have been of major significance, but is marred by dogmatic classification of specific ore deposits—such as the Red Sea as intra-continental rather than an incipient ocean rise, the southern European Permo–Triassic deposits as a trailing edge, and so forth. This may all be correct but is certainly arguable, yet the final conclusions are quoted to 0.01%; and one major environment, with 37.69% of world reserves, occurs in trailing margins—an environment which seems to be defined as one into which sedimentary ores are placed together with those which are not obviously in any other category. The conclusion should be that some one-third of the world's lead occurs in sedimentary forms of uncertain origin.

The general lack of consideration of sedimentary ores is understandable, although disappointing. There is evidence that most of these have been strongly influenced by palaeoclimatic conditions and hence palaeolatitude. As this is determined by the motions of the plates, these ores are as related to plate tectonic motions as subduction zone metals. Other gaps, however, include kimberlites, which are not merely important economically but have major implications for upper mantle evolution. Nevertheless this publication is obviously essential to even the smallest geological library. The hope is now that some future NATO conference will turn to consider energy resources in the same context. The seafloor conveyor still has some way to go before all of the ideas have been sweated out of its subducting melange! **D. H. Tarling**

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Opisthobranch molluscs

British Opisthobranch Molluscs. (Synopses of the British Fauna, No. 8.) By T. E. Thompson and G. H. Brown. Pp. 203. (Academic: London, New York and San Francisco, 1976.) £3.50.

This is a very welcome addition to the recent series of Synopses of the British Fauna. The book is well designed and attractive to look at. There is less restriction on size than in previous numbers and this has enabled the authors to have, for most species, a large drawing occupying one page and on the opposite page a brief but adequate description of external features necessary for identification, notes on biology and worldwide distribution. Technical terms have been kept to a minimum and the introductory pages and descriptions are designed to cater for the non-professional zoologist.

The complementary talents of both authors are well exhibited. Gregory H. Brown's drawings set forth the salient features of each species and the life-like posture. T. E. Thompson, a well known specialist on opisthobranchs and an aqualung enthusiast, is familiar with the habitats of many species and has examined nearly all the 135 which are described. When opisthobranchs have to be identified it is now no longer necessary to search the classical works and paraphrase the language of nearly 150 years ago. In this synopsis concise keys lead to family and species. Ideally such attractive molluscs would be depicted in colour, but the cost is prohibitive. The single plate that is provided, although valuable in displaying their beauty of form, whets the appetite for more.

In such a detailed compilation it is difficult to prevent a few misconceptions: the eyes of *Stiliger bellulus* are lacking in the description and the figure; the dorsal surface of *Limapontia capitata* is figured with a pale cross, but this is of rare occurrence.

V. Fretter

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nature February 10, 1977

LIBRARY C.U.

Tidal project worth support

SITTING somewhere in the Department of Energy is a proposal by a team of engineers known as the Severn Barrage Group for a major study of the feasibility of extracting substantial amounts of tidal energy from the flow of water in the Bristol Channel/Severn Estuary region. The outcome of the request for £1.2 million to do 18 months' investigative work will be watched with great interest by all who have a concern that no energy option should be closed out without some pretty sophisticated analysis and without an extended time being available for public debate.

Tide mills are a pretty old concept. T. L. Shaw, writing on the Severn scheme in *Nature*, **249**, 730 (1974), remarks that they can be traced back to 1180, and the idea of harnessing the unusually high tidal range in the Severn Estuary (mean range 7 metres) was first given detailed engineering study as far back as 1933. Even when nature conspires to provide as favourable a site as the Severn Estuary, however, everyone knows that tidal energy suffers from the severe defect that if turbines are simply driven by the flow of water into and out of the estuary, the periodicity of the daily demand for electricity is only spasmodically in phase with the times at which tidal power can make a useful contribution. There are enough thermal generating sets around the country suffering reduced efficiency because of this daily demand cycle for there to be little enthusiasm simply for the addition of another cyclic term, and, what's more, one with a 29-day spring/neap periodicity to bedevil operating conditions even more. It is for these reasons that the French, who have successfully run a 240-MW tidal generator at La Rance, Brittany, for many years, have never proceeded to the larger scheme for which La Rance was the pilot.

The Severn Estuary scheme as now proposed would, however, not be tied to delivering energy in phase with the tides, but would be part of an integrated scheme providing pump-storage facilities for conventional thermal stations. A barrage would close off the estuary between Cardiff and Weston; above it would be the primary basin in which the water level would fluctuate between the present mean sea level and the high spring-tide level—that is, there would be no flooding of land. Just seaward of the barrage, in deep water, would be a secondary basin, maybe 5 kilometres across. Water from this basin could be pumped up into the primary basin using electric power from thermal power stations. Electricity would be generated both by the fall of seawater at high tide into the (drained) secondary basin and also by the fall of water in the primary basin back into the sea at low tide. Electricity would be generated during the daytime and consumed in pumping at night. In this way a scheme combining tidal energy extraction and pumped storage would take electricity from thermal stations at times at which they might otherwise have to close down.

Rough estimates put the cost at £2,000 million to generate around 4,000 MW during the day (nearly a tenth of the nation's load at present)—in itself rather an expensive investment, though less so when the advantages of the pumped storage scheme are incorporated and consideration is taken of the price, or lack of it, of fuel.

Until now studies on the Severn Barrage have been conducted at a fairly academic level, and only recently has much serious work been done on the obviously major environmental impact; a cursory examination of material published suggests no overwhelming environmental objections. The time looks ripe therefore for a much more extensive feasibility study. Around £1 million has recently been committed to exploratory research for geothermal energy sources which seem less likely to make a major impact on the nation's energy requirements; a similar sum on tidal energy and the effect that the scheme could have on the environment does not look to be out of line.

If a study is funded and finds that the case for some sort of barrage is strong and that environmental objections can be met, there is still bound to be one major question mark hanging over the project. Once into such a scheme there is little benefit gained in calling it off on the way to completion. If costs go awry, if major problems arise, if the energy scene changes dramatically, there is little that a half-completed barrage can be used for. This will doubtless be used as ammunition by those who have a distaste for large technological projects, so it would be more than usually necessary that both the feasibility survey be done exceptionally well, and that the politicians who make the ultimate decision be thoroughly open with the public about the basis of their decision—since they probably won't be around at the time when nerves are being tested. ☐

Keeping in touch with Soviet colleagues

Miles Reid, Royal Society Visiting Fellow in the Department of Mathematics at Tokyo University and Fellow of Christ's College, Cambridge, writes an open letter to scientists interested in maintaining and improving contacts with their Soviet colleagues

THE growth of international scientific and cultural ties is potentially of enormous value in developing the spirit of cooperation and friendship between nations. I personally believe we scientists can make a contribution towards making this world a safer and a more decent place to live in. But it is also my opinion that Western scientists do not make full use of existing channels of communication, perhaps due to traditional western misinformation as to what is or is not feasible.

Invitations to the West. The majority of Western scientists are probably familiar with the frustrating fact that senior (non-party) Soviet scientists invited to Western conferences find it impossible to take up the invitation.

The first step for any Soviet citizen in obtaining a visa to travel abroad is to get a reference from the Party Committee of his place of work recommending the visit; there are several subsequent steps where hitches may occur, but I believe the first step to be the essential obstacle, since if the *Partkom* is prepared to give its backing the remaining problems may turn out to be soluble.

Now there is some reason to believe that this obstacle may be less serious for younger scientists. This optimistic view is suggested partly by analogy with the case of visits from the USSR to the countries of eastern Europe (a closer analogy than most people in the West will be aware of); and partly by speculation as to the nature of the decision-making procedure within the *Partkom* of universities and institutes.

I would thus like to urge that every possible opportunity be taken to invite the younger generation of Soviet scientists to meetings in the West or to Western universities. In my field of mathematics (algebraic geometry) there is certainly no shortage of excellent young scientists, and visits to the West by such people, even for short periods, should provide for an extremely stimulating flow of ideas and information in both directions.

I would like to suggest that as a matter of form every invitation to a senior scientist should contain a clause to the effect that if the person invited feels unable to take up the invitation, he suggests the name of a junior colleague or research pupil who could be interested in coming in his stead. Of course, one does not necessarily expect that a research student of a particular scientist would be able to do more than give a token representation, but his presence will allow a flow of ideas, and will do something towards correcting the balance in the make-up of Soviet delegations.

As far as I am aware almost any young Soviet scientist would jump at the opportunity of travelling to the West, and be extremely keen to attempt the considerable task of applying for permission. There is probably a practical upper bound of about two months on the length of time a Russian scientist would be allowed out, and one should allow for some uncertainty in the time of arrival.

> 'It seems to be the case that Western organisers who have had repeated failures in attempts to invite Soviet colleagues sometimes get discouraged and give up trying. This is obviously wrong on probabilistic grounds'

Contrary to what is commonly supposed in the West, there is no reason of principle why a Soviet citizen having obtained permission to travel abroad should not support himself on the issue of dollars he is entitled to under the currency regulations, although I am informed that university authorities may be very much more impressed by an invitation promising support and even a contribution towards travel expenses.

Even for a (non-party) scientist with an impeccable student and *Komsomol* background the probability of being allowed out is probably no greater than one-tenth, and it seems to be the case that Western organisers who have had repeated failures in attempts to invite Soviet colleagues sometimes get discouraged and give up trying. This is obviously wrong on probabilistic grounds, since if the probability of success is small one should clearly make more attempts rather than fewer. And if one draws a blank after taking reasonable measures over a period of several years (such as writing periodic supporting letters to individual members of the university or institute management urging the advantages to both Western and Soviet science to be gained by some particular visit), then one is in a stronger position to complain that the Soviet side is being uncooperative.

There is certainly no way in which invitations addressed to an individual scientist, or letters enquiring as to the best way of inviting either him or one of his students could compromise him or lead to retaliation from the authorities.

I regard suggestions sometimes made that we take a hard-line attitude and boycott delegates we regard as too 'official' as potentially extremely harmful and counterproductive. It is my firm conviction that success in this matter will be directly proportional to the amount of patient effort which we are willing to exert.

The copyright convention. In 1973 the Soviet Union signed the Geneva copyright convention, thus bringing to an end the tradition of publishing pirate translations of Western books and journals. The potentially damaging effect of this on Soviet mathematics doesn't seem to have been generally realised. Before the copyright convention any important textbook was published in an edition of 10,000 (say), ensuring that every college, university or technical school was able to obtain a copy for its library. Similarly important Western articles found their way into libraries across the country in the form of translations in *Matematika*. Since the convention the number of textbooks translated has dwindled to an insignificant trickle, and *Matematika* has ceased publication.

Important university or institute libraries get an issue of foreign currency with which they can buy a selection of the essential literature; but this may have the effect of reducing contact with Western scientific ideas down to the scientists at a few major centres. An important additional source of foreign journals costing nothing in dollars is the exchange of publications, and every attempt should be made to extend the existing exchanges. The use of the photocopying machines at the major Soviet libraries remains at present expensive and inconvenient.

Contact by mail. A visitor at a Russian scientific centre is constantly embarrassed by being pumped for information about what's going on in the West; and the plea for preprints, lecture notes and copies of letters is invariably repeated in any discussion. I am not necessarily implying that the Western scientific community has been mean in this respect in the past, but I'd like to emphasise the desirability of sending as much information as possible.

Although it is true that every item of news received from the West is circulated and discussed with great interest, it is also true that even within Moscow there are many different institutes at which scientists are employed, and there may not be very close contact between them; *a fortiori* this applies to provincial universities. Western scientists need only consider how frequently they make photocopies of preprints or letters for their own use or for passing on to a colleague or student to realise the inconvenience of being deprived of this facility; so it doesn't seem unreasonable to suggest sending a larger number of preprints to a Russian university than one would to the corresponding Western university.

It is perhaps quite striking the extent to which the average scientist depends for his general outlook on informal contact. Thus one might discuss in conversation the work in progress of some colleague and gain thereby an overall impression of some new field of research without having to wait for the precise results to appear as preprints or articles. For a while now I have been trying to apply this principle in written form to try to bridge the gap between Soviet mathematicians in my field and the West, writing with occasional news of new developments (sometimes of which I have only the sketchiest knowledge myself). A slight difficulty is that there are in principle and in practice restrictions on one's correspondents' replies (see below), and one sometimes has to content oneself with the abstract knowledge that the communication is well received, and somehow get over the psychological difficulty of corresponding with someone whose replies are infrequent.

This idea has a potentially invaluable application to an international gathering at which no Soviet delegate is present, and I would like to suggest that if maybe two or three of the participants write their impressions of a few of the most exciting new results, and send three or four copies to interested colleagues in the Soviet Union, then this will do something towards correcting the isolation of Soviet science.

This problem has a converse: Russian scientists also complain that it is very difficult for them to send round copies of their recent work; some of the controversy about Russians not getting fair recognition for their original work perhaps stems from this difficulty.

An article prepared for publication in a Soviet journal will be typed, with three or four carbon copies made, of which several have to be submitted. It would be useful to have some system whereby a Soviet author who so desires could send out a carbon copy of his typescript to be copied and distributed in the West. This could perhaps be done officially, for example in connection with the translation schemes, or unofficially by private arrangement; those of us who are in correspondence with Soviet scientists should at any rate offer to perform this function.

> 'It would be useful to have some system whereby a Soviet author who so desires could send out a copy of his typescript to be distributed in the West. This could perhaps be done officially, for example in connection with the translation schemes, or unofficially, by private arrangement'

There are in principle two legal restrictions on what can be sent out of the Soviet Union. Firstly, the State holds the copyright on anything written by one of its citizens. And secondly an archaic clause of the State Secrets act requires that before sending abroad any written item containing mathematical formulae the sender must obtain the explicit permission of a scientific establishment—which involves the completion of exhaustive formalities, and is usually only possible if the item is already cleared for publication. In practice, however, scientific manuscripts and short items containing formulae are sent abroad rather frequently, as shown for example by the fact that Soviet scientists review regularly for Western journals and translate for the AMS. In general I am impressed by the amount of material that has been permitted in recent years to pass through the mail in both directions, and I would like to interpret this as an indication of the genuine application of one of the clauses of the Helsinki agreement.

It could be argued that the postal regulations, which make it impracticable for papers submitted to Soviet journals to be sent to a reviewer outside the USSR, are partly responsible for the uneven quality of some of the papers appearing in the Soviet scientific press.

Visits to the Soviet Union. The possibilities are as follows: as a tourist; by formal invitation; under the international Cultural Exchanges.

- It is perfectly feasible to visit, say, Moscow or Leningrad as a tourist, and nowadays the tourist gets a very reasonable deal (travelling with a group may be very much cheaper, and the group activities are not compulsory). When applying for a visa one is required to name the Soviet citizens one intends to meet, but there can be no objection to a scientist who intends to get in touch with colleagues declaring only one name.

It is perhaps worth pointing out in connection with the forthcoming International Congress of Mathematicians that Leningrad is just one night on the train away from Helsinki, and is a town that has considerable attraction to the tourist.

- Soviet scientists may be very willing to attempt to get an invitation made out to a Western colleague to visit them for periods of up to two months. However, it is fair to say that this is not necessarily straightforward. Certainly any Western scientist interested in this possibility should in the first instance write well in advance to a Soviet colleague with whom he has good scientific relations to discuss tactics.
- There are cultural exchanges in operation between the Soviet Union and every Western country. They take place both at the level of visiting fellowships (organised through the Academy of Sciences) and visiting studentships (through the Ministry of Higher Education), and represent almost the only practical possibility for a Western scientist to spend a long period (six months or a year) in contact with a Soviet scientific centre.

I would strongly recommend the latter to any Western research student of recent PhD who is interested in some aspect of Soviet science, is willing to learn Russian, and is prepared to face the difficulties as well as the varied delights of Soviet life. Copies of my report to the British Council containing some of the fruits of my two years' experience in the USSR will be available on request. The very least that Western universities can do to encourage the use of these exchanges is to display the advertising matter relating to them.

In conclusion I would remark that there are many countries in the world where the working conditions of scientists, and the ways in which contacts with the world scientific community can be improved, are subjects which certainly deserve further study. But it seems fair to say that the Soviet Union, the People's Republic of China and Czechoslovakia stand out as the three major countries of the world whose minimal cooperation in maintaining scientific contact amounts to a deliberate affront to the world academic community on the part of their governments. ☐

Three years after Allende

Alwyn Eades, recently in Chile, assesses the state of the physical sciences there

DURING the past three years, research in physics in Chile has suffered set-backs from which it will recover only with difficulty. University physics departments have been especially hard hit; they have no protection from the severe budget reductions and loss of staff brought about by the political upheavals. In materials science the position is less bleak and people are hanging on, if only by the skin of their teeth. This is because the centres of applied science have partial independence. The Institute of Materials Testing and Research of the University of Chile, for example, is in a stronger position than the physics departments because it is able to pay (and has to pay) the whole of its research budget from contract work. Only staff salaries are paid by the University.

This reversal is in marked contrast with the previous steady growth of research. Twenty years ago there was little or no research in physics in Chile. Then in the mid-1950s a small number of physicists, trained abroad mainly in England, began to set up research programmes. This effort was initially slow and precarious, but by the late 1960s they had met with such success that the two most important physics departments in the country (both in the University of Chile, one in the Engineering School, the other in the Faculty of Sciences) each had about 40 academic staff including graduate students, nearly all of whom were involved in research.

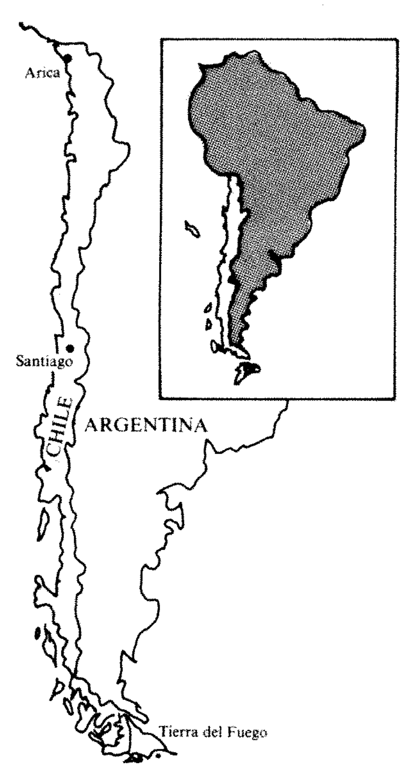
At this point it began to seem for the first time that there were enough research physicists in Chile to form a stable nucleus for future development, and that some kind of critical mass had been achieved. Evidence for this lay in the establishment of a doctoral programme; until that time Chilean students were obliged to go abroad to get a higher degree. In the early 1970s people felt such confidence in this stability that there was a lot of talk of developing a science policy for Chile in a way which was unthinkable previously. For the first time, Chilean physicists began to feel secure that no longer could an arbitrary administrative decision or the departure of one man have a disastrous effect on the nation's research effort. It was therefore bitterly ironic that the military coup of September 11, 1973 should, almost incidentally to its many brutal acts, set physics research back nearly two decades.

It is worth pointing out here that the election of Allende did not directly affect science in Chile very significantly. The presidency of Salvador Allende represented much more of a continuation and natural extension of previous government policy in Chile than is usually recognised outside the country. In any case, the principal effects on science research were, initially, considerable governmental encouragement to make research relate to problems relevant to Chile and, later, a reduction in the budget available in hard currency. The latter was a result of economic problems rather than an attempt to restrict science.

The fate of the two physics departments mentioned above since the coup has been quite different. The Department of Physics in the Faculty of Sciences lost most of its staff within a very short time of the coup. Many left voluntarily to seek employment in other countries. Some were imprisoned and/or expelled. In the three years since the coup there has been a bare handful of staff and there has been no research. I was told, for example, that the two main experimental areas, the cyclotron and a low temperature facility for solid state research, have remained closed down. In the past few months, however, new staff members have been contracted and attempts made to restart research. It is surprising to find that those involved seem confident that stable conditions suitable for research may now prevail.

In the other physics department, that of the Engineering School—a right-wing stronghold in a largely left-wing university—the coup had little immediate effect. My own departure two months later was one of the few obvious results. Over the three intervening years, however, the situation has deteriorated very greatly. There have been several waves of sackings for a mixture of political and economic reasons, as a result of which the number of academic staff has dropped from 45 to about 25. Many research groups have completely vanished.

For example, a very impressive semiconductor and microcircuits facility, set up with substantial funds from the Organisation of American States and the Ford Foundation with the support of the Chilean Development Corporation, now lies virtually abandoned. One of these two laboratories is a complete manufacturing facility for microcircuits; the other is a well equipped laboratory for basic research into semiconductors with no one to use it.



Chile is a large, sparsely populated country. Three million of its ten million inhabitants live in Santiago, the capital city, where virtually all economic activity except mining and agriculture is concentrated.

A former Spanish colony, Chile gained her independence at the beginning of the nineteenth century. Subsequently she maintained a remarkable record (indeed a record unique in Latin America and scarcely matched anywhere) for unbroken democracy and liberal government. She was, for example, among the first countries to abolish slavery, and her social services were very well developed for such a poor country. From the 1930s consistent policies by successive governments succeeded in achieving a substantial degree of industrialisation, though mostly with production on a rather small scale and using imported technology.

The country's stability was broken in September 1973 when a military junta overthrew the leftist government of Dr Salvador Allende, who was killed. In the years since the coup the junta has followed a policy of repression and infringement of human rights, and an economic policy which has caused both a large reduction in production with many companies both large and small going bankrupt, and very high unemployment.

Alwyn Eades, who wrote the accompanying article, is at the University of Bristol and worked in Chile from 1967 to 1973. He recently returned there for the first time in three years to attend a scientific congress and work with his former colleagues. He spent two weeks in the Department of Physics of the Engineering School and visited four other institutions. Together the five laboratories represent a very large proportion of the nation's research effort in physics, materials science and related areas. "It is very easy to be misleading about Chile", he says, "and very easy to be misled".

This particular state of affairs is still more disconcerting for another reason. Chilean physics has been characterised by too many groups of too few people covering too wide a spectrum of research; moreover, research topics have been chosen because of the training received by staff abroad rather than because of any relevance to Chile. The now-abandoned semiconductor facility was the beginning of an attempt to change this.

The pattern occurs elsewhere. Thus the plasma laboratory, equipped with a well instrumented shock tube and once with six staff members, is now deserted. The only remaining member of the group is completing postgraduate study in London University and is reluctant to return to work alone instead of rejoining an active group with research in progress. Laboratories for Mössbauer spectroscopy and nuclear magnetic resonance also lie idle.

Some groups are not so unfortunate, and in particular X-ray crystallography, biophysics and theoretical physics remain active. The first is particularly important because Oscar Wittke and his team not only do good crystallography but also carry out invaluable service work for industry. This long standing work is unique as an example of collaboration between industry and a physics group in Chile. But even these more fortunate groups are building up problems for the future because of their stagnation and the lack of young people receiving training. They are also handicapped by an arbitrary and inefficient administrative structure and erratic finance. This is not new, of course, but it is an increased handicap when conditions are precarious for other reasons.

Three non-university research laboratories have had mixed fortunes. CIMM is the Centre for Research in Mining and Metallurgy. It is financed by the national Copper Corporation, and since copper mining plays such a dominant role in the Chilean economy, CIMM enjoys a privileged position. In the laboratories the equipment is in use and a large research team is not only providing a service to the mining industry, it is also pursuing a number of exciting original lines of research. I gained the impression that recent events have hardly affected them. One of the very few foreign research workers to enter Chile since the coup, John Burley, an Englishman, is helping to build up the microscopy section.

Two other institutions—INTEC (The Technological Institute) and CESMEC (The Centre for Testing and Quality Control)—have been less fortunate. They were set up by the United Nations Development Programme (UNDP) which provided the basic facilities and equipment and were subsequently financed by the Chilean Development Corporation (CORFO). In the three years since the coup there has been no direct interference in the running of the laboratories, perhaps because of the quasi-international status deriving from their UN association, but the financial support from CORFO has been cut.

In the case of CESMEC it has been withdrawn completely and they have been forced to become entirely self-supporting from the contract work undertaken for industry. This has necessitated substantial reductions of staffing levels and all projects other than the contract work itself have been abandoned. Moreover, they are functioning entirely on capital, in the sense that they are using the equipment provided by UNDP with little possibility of proper maintenance or purchase of spares and none at all of purchasing new equipment.

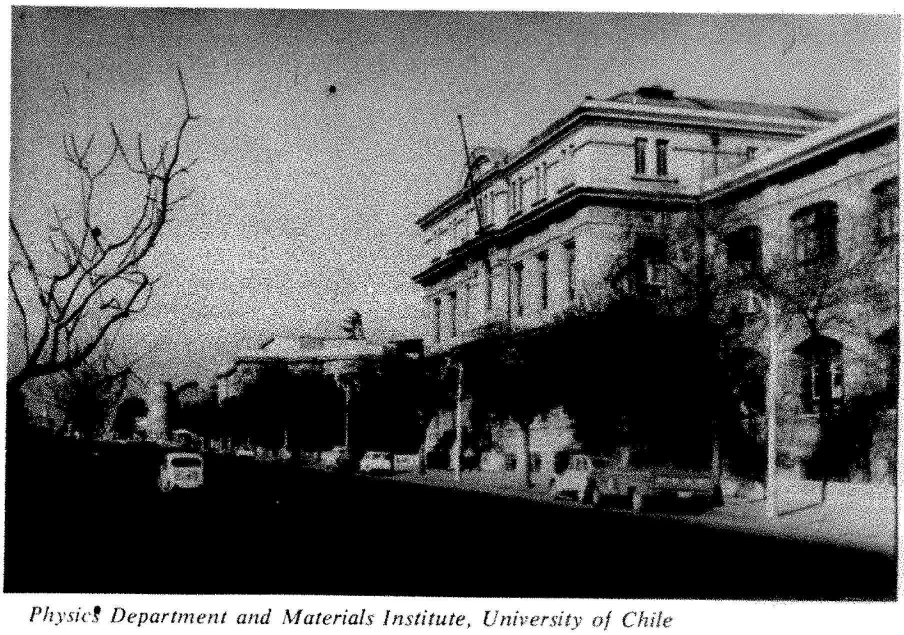
The future of both institutions is very precarious. It is possible for contract research organisations in the developed world—Battelle Labs in the USA, for example, or the Fulmer Research Institute in Britain to be successfully self-supporting—but to expect the same in a semi-industrialised country like Chile is a pipe-dream. Nonetheless, both organisations are doing good work and playing an important role in the industrial development of the country. If government financial support is not restored, both organisations may not survive more than a year or two—another reversal of progress in the country which will bring the return to a time when all expertise has to be bought outside the country; even for certain kinds of routine analysis, specimens will once again have to be sent abroad.

CIMM, CESMEC and INTEC were all founded within a decade, which might be a surprise since they now contribute so much to the mining industry which is the mainstay of the economy. However, it is only recently, a century and a half after political independence, that colonial attitudes have faded sufficiently that Chileans have begun to recognise that their own expertise can be as valuable as that purchased overseas.

Repression remains severe in Chile, but in many ways the country is back to normal and on the whole people are not nervous about discussing the situation in an open way. 'Normal' in this context means that there are no longer heavily armed police at every turn, nor military vehicles conspicuous in the streets; despite the continuation of the curfew there is once more a normal, if curtailed, night-life and the public does not in general, show signs of tension or strain. It would be possible for a tourist to get no hint from what he sees of how things are below the surface.

The recent release of prisoners held without trial under the "State of Siege" legislation, however, does not mark the end of repression. Those people who are taken prisoner now are taken in secret and the authorities do not admit to holding them; this is, of course, worse than before. Although I heard of a number of such cases, I am not aware of any scientist who is held prisoner now.

Unemployment in Chile has been extremely high since the coup. Even on official figures it now stands above 15% and is probably higher. But this figure hides enormous variations. An international official, who knew from first-hand experience in voluntary social work, said that in some working class areas unemployment runs at 90%. On



Physics Department and Materials Institute, University of Chile the other hand there seems to be very little unemployment among the professional classes. The very extensive sackings and cut-backs in all government departments as well as in private industry have reduced employment, but amongst professionals there seems to have been enough mobility and opportunity for them to move overseas. The ex-Dean of the Engineering School was reported as saying that of 11,000 Chilean engineers, 4,000 are now in Venezuela.

The Chilean Physical Society (SOCHIFI), under its president Claudio Gonzalez, continues to function and hold its scientific meetings in the usual way, although with considerable difficulty because of the loss of members and reduced financial support. One of its present preoccupations is that, since all elections are prohibited in Chile, it is unable (like many other organisations) to replace those committee members who leave the country or retire. It may shortly find itself with no committee at all. □

USA

Overheads: a growing problem

Colin Norman reports from Washington on a growing dispute involving overhead costs associated with government-funded academic research

THE federal government in the United States will spend about $3,000 million this year to support research and development in colleges and universities. According to one widely accepted estimate, however, between $600 and $700 million of those funds will be taken up by overhead costs such as heating and lighting, university libraries, departmental administration and so on.

The size of those costs has long been a source of irritation between researchers and university officials, and between the universities and the federal government, but there are signs that the irritation may develop into a serious dispute when the budgets of some research agencies are considered by Congress later this year. Moreover, the universities have been expressing considerable concern about a review of overhead costs which has been under way in the Executive branch for the past 18 months. Important principles underlying the support of research in the United States are involved, and university officials are now preparing for battle.

It is easy to see why concern about overhead costs is growing. Overheads—which are usually referred to as indirect costs—have been rising faster than the direct costs of doing research in the past few years, which means that indirect costs have been taking up a steadily increasing share of the federal support for academic research. Since, until very recently, total federal funds for basic research have been declining in purchasing power, university scientists are alarmed that the universities are taking an increasingly large bite out of already scarce resources.

And there seems to be a feeling among conservatives on Capitol Hill that the universities may be using federal research grants as a way to obtain revenue to support a variety of questionable activities. A staff aide to one right-wing Congressman argued in an interview last week, for example, that "the taxpayer is getting ripped off because the universities are turning basic research into a porkbarrel" for higher education. Indirect costs, he opined, are "a giant subsidy to the universities".

Such sentiments naturally touch a raw nerve among university administrators who are already grappling with steeply rising costs and diminishing federal support. They generally argue, however, that much of the criticism is based on a misunderstanding of the nature of indirect costs and the manner in which they are calculated. Though a number of extremely complex accounting procedure are involved, the basic concept is relatively simple. Procedures differ from one institution to another, but it generally works as follows.

When a researcher applies for a grant for a particular project, he calculates the direct costs of salaries, equipment, technicians' time, computer use and so on. The university then adds a fixed percentage for overheads, and the application is submitted to the federal government. If the grant is awarded, the university keeps the overheads and passes the funds for direct costs to the researcher.

The indirect costs are supposed to cover items and services which are shared by a number of research projects and which cannot be charged to any individual grant. Each university calculates its own indirect cost rate each year, but it is allowed to include only certain items specified by the federal government in a document known as FMC 73–8. The rate charged by the university is subject to audit to ensure that no unallowed costs are included. The rates differ from university to university, with the private universities generally charging the highest overheads and the large state universities, which receive some state subsidies and where economies of scale apply, charging lower rates. At Stanford, for example, the rate is 58%, which means that for every $100 required for the direct costs of a research project, the university adds $58 to the grant application to cover overheads.

A good deal of confusion has arisen because different universities express their indirect cost rates in different ways, with some expressing it as a percentage of researchers' salaries and others as a percentage of total direct costs, but there is one inescapable fact: however they are expressed, indirect cost rates are increasing sharply. According to a Congressional staff investigation published last year, for example, the average overhead cost rate has increased from about 30% in 1970 to about 35% in 1975. At Stanford, the rate has gone from 47% to 58% in the past six years.

The universities can cite good reasons for the increases. Charles V. Kidd, executive secretary of the Association of American Universities, notes, for example, that a number of recent federal regulations, such as equal pay requirements, privacy legislation, occupational safety and health regulations and so on, have placed considerable cost burdens on the universities, and many items, such as fuel costs, have been rising particularly steeply. According to William F. Massey, Vice-Provost of Stanford, for example, the university's fuel bill alone has climbed from $800,000 to $5.5 million in four years. Moreover, Kidd argues that because of the general financial squeeze on the universities in the past few years, institutions have been more diligent in claiming their legitimate costs from the government, and that has tended to push the cost rates up.

Nevertheless, in response to concerns over rising indirect costs, the Department of Health, Education and Welfare (HEW), which is the largest federal sponsor of academic research, has been reviewing the ground rules governing indirect cost rates. In July 1975, HEW proposed a number of revisions to FMC 73–8 which would have placed severe restrictions on the types of services which the universities could claim as indirect costs. Under the HEW proposal, for example, the universities would no longer have been able to include the costs of libraries in their indirect cost calculations, and some administrative expenses would also have been excluded.

The universities complained bitterly, and last month HEW revised the proposals to make them a little more palatable. The proposed revisions are now under consideration in the Office of Management and Budget, and it will be several months before any formal changes, if any, are made to FMC 73–8. If any revisions are made, they will inevitably restrict the amounts which can be claimed by the universities.

There are indications, however, that some stiff criticisms of indirect research costs may break out on Capitol Hill this year. Some of the Congressmen and Senators who in the past few years have poured scorn on research projects with trivial-sounding titles and who have criticised the National Science Foundation (NSF) for its grant review procedures, are beginning to take a critical look at the increasing indirect cost rates. Thus, for example, Robert Michel, a Republican from Illinois who was instrumental last year in blocking funds for a behavioural science project which he found distasteful, questioned the rapid escalation of indirect costs in NSF grants when the agency's budget was before Congress last year. He indicated that he would take a closer look at the matter this year. And George Archibald, a key staff assistant to former Representative John Conlan, NSF's chief critic in recent years, is now administrative assistant for Conlan's successor and has already begun to investigate the matter of research overheads for his new boss.

Some of the criticisms are likely to surface when a subcommittee of the House Committee on Science and Technology holds a public hearing on indirect costs on February 22. And the House Appropriations Committee is also expected to probe the matter when the budget for the National Institutes of Health is under consideration.

Few observers here are willing to predict the outcome of these various executive and legislative investigations, but one veteran science policy watcher, now working for the White House, notes that skirmishes over indirect costs have erupted at various times since the federal government became a major sponsor of academic research in the second World War and he speculated that this skirmish will follow the familiar pattern: there will be considerable argument, the federal ground rules will be tightened and the dispute will die away until the next skirmish arises.

This time, however, there is a potent combination of irritants arising from the steep increase in overhead rates, the serious financial situation of many universities, and a strong desire among fiscal conservatives in Congress to seek ways to cut the federal budget. The dispute goes to the heart of the system by which the federal government supports university research, and it is thus of considerable importance to the overall health of American science. □

Taking politics out of health

Keeping an earlier pledge to "de-politicise" the National Institutes of Health (NIH), Joseph A. Califano, the new Secretary of Health, Education and Welfare (HEW), last week paid a visit to the NIH campus to announce that the Carter Administration has decided to retain Dr Donald S. Fredrickson as NIH Director. The announcement, delivered to a packed audience of research workers, was included in a gentle pep talk in which Califano again promised to insulate NIH from partisan politics, and gave assurances that he would personally seek more resources for biomedical research.

The announcement, and the fact that Califano went to NIH to deliver it, should help to soothe complaints from biomedical researchers that NIH has been subjected to unwelcome political pressure in recent years. During the Nixon Administration, for example, candidates for NIH advisory committees were screened for their political affiliations, and two NIH directors have recently been fired, reportedly for political reasons. The first, Dr Robert Q. Marston, was dismissed by Mr Nixon in January 1973 for opposing budget cuts and the elimination of some cherished training programmes, and his successor, Dr Robert S. Stone, was fired by Mr Ford in January 1957 for alleged administrative incompetence.

Dr Fredrickson, a well respected scientist and administrator, made known his desire to stay on as NIH Director, but there was widespread speculation over whether the Carter Administration would retain him or replace him with a political appointee. In announcing the decision to keep Fredrickson as Director of NIH, Califano said that the Administration had looked "only for excellence" and he had not asked any candidate for a post in HEW about his or her political views. Frederickson, he said, had been selected because he is "the best person to do the job that has to be done".

Although Califano's remarks went down well with his audience, he himself acknowledged that the problems which have been afflicting NIH in recent years cannot be cured simply by ending the political screening of candidates for NIH posts. NIH has been suffering from a combination of budgetary stringencies and rapidly shifting priorities as funds have been channelled into cancer research and, to a lesser extent, heart research at the expense of some other programmes. To a large extent, those shifts in priorities have resulted from legislation which has given the National Cancer Institute a privileged political status in NIH. And there has also been a trend which has resulted in a growing proportion of NIH funds being disbursed as contracts rather than grants—a trend which has increased the central management of biomedical research, but which has met with stiff resistance from academic scientists.

In response to some of the concerns which have been expressed in the past few years about the growing politicisation of NIH and the disparities in funding, Congress established a Presidential commission under the chairmanship of a former medical school dean, Franklin Murphy. The Murphy panel last year issued a number of recommendations designed to reduce the privileged status of the cancer institute and to ease the strains within NIH. The Ford Administration made no move to implement the proposals, however, and the question now is whether or not Califano will act on them.

As for funds for NIH, although they have been increasing in the past few years, they haven't kept pace with inflation. According to an analysis recently completed by NIH's budget office, for example, the proportion of research grants approved by peer-review committees which have actually been funded has declined from 58% to 35% over the past three years, and it will drop to 32% next year if Mr Ford's final budget proposals are implemented. Califano said, however, that "I believe the work you do here, and that basic research generally in health, is critical for our society and needs additional support and resources, and I'll do my best to try and get them for you".

That went down particularly well with his listeners.

Colin Norman

SEVESO

Seven months on

Alastair Hay updates developments since the Seveso disaster in northern Italy last year

SEVEN months after the explosion in the trichlorophenol reactor at Seveso which resulted in the release of tetrachlorodibenzo-*p*-dioxin (dioxin), more details of its consequences are becoming apparent. The large number of organisations involved in assessing damage and formulating policy for decontamination of the area has prevented a crash programme. There has, however, been considerable agreement as to a general plan of action—a 'clean-up' operation to try to remedy damage, the full extent of which is still unknown.

According to Mr Alex Rice of Cremer and Warner—the British firm of consulting chemical engineers acting as 'technical auditors' of the decontamination programme—the work of removing dioxin

> "is proceeding, and there will be a high degree of reclamation of property and land in a reasonable period of time. The nature of the discharge of the reactor contents resulted in a differential degree of dioxin contamination in different areas. It has been necessary, therefore, to arrive at a graded response to solve the problem of decontamination.
>
> At the moment, in the most severely contaminated area designated as 'Zone A', hard surfaces are being cleaned and it is hoped that some 80% of the residents evacuated from the area will be able to return to their homes in a few months. Meanwhile, in 'Zone B', less severely affected by dioxin and where inhabitants have been able to remain in their homes, vegetation in certain selected areas is being cut down and incinerated".

From the evidence available at Seveso, Cremer & Warner estimate that 2.5—3 kg of dioxin was released in the reactor discharge. On this basis and through information obtained from a model simulating the explosive reaction at the ICMESA chemical plant, they suggest that only a few hundred grams of dioxin was actually deposited on vegetation and other surfaces. They maintain that the majority of dioxin was too finely dispersed and in a particulate form too small to permit precipitation to occur, with the result that thermal air currents carried the material high into the atmosphere. Conditions on the day of the explosion were ideal for the photochemical decomposition of dioxin at these altitudes, but there is no evidence to substantiate that such a degradative process actually occurred.

Earlier reports suggested that the dioxin was percolating through the soil at Seveso more quickly than recorded on previous occasions in different localities. More recent investigations, however, confirm that 98% of the dioxin is strongly adsorbed on the top 4cm of soil. Some of the methods of soil sampling in the earlier stages of the decontamination programme were not rigorous enough to exclude contamination of lower layers by material on the soil surface. The later investigations confirm previous reports in the literature that dioxin is relatively immobile in soil.

The question of the teratogenic properties of dioxin is still causing considerable anxiety to women in Seveso. The Lombardy regional government ruled last August that pregnant women from the contaminated zones around Seveso could have legal abortions, but local doctors have in many instances consistently refused to terminate pregnancies, and only a small proportion of pregnant women from Seveso have had abortions in Italy. The foetuses from these women are currently being examined for any signs of abnormalities and the results of the investigations should be available by March.

The reluctance of the Italian doctors to perform abortions has forced many Seveso women to seek a termination in other countries. It is doubtful whether any of the foetuses from these abortions will be examined for malformations. Doctors in Britain have voiced their concern over this loss of potentially valuable information on human teratology. They argue that there should be some agreed international code of practice on the matter to prevent it recurring and add that in Britain in particular there are arrangements to facilitate abortions and examination of the aborted foetuses for signs of abnormalities.

In the next few months women from Seveso who were in the first trimester of pregnancy in July last year will reach full term. The children born to them and to the mothers who conceived after the explosion are those most at risk from the teratogenic properties of dioxin. It is proving an agonising time for these mothers as no one can reassure them that their 'dioxin children', as they are known locally, will be unaffected. For them, as for all the people of Seveso, certain reassurances are impossible; it is just a matter of waiting.

- A report in the Turin paper *La Stampa* this week says that many children at a school in the Seveso area are suffering from skin rashes.

IN BRIEF

European energy policy moves

Several important aspects of European energy policy were discussed in talks in London last week between Mr Anthony Wedgwood Benn, the UK Energy Minister, and his West German and Dutch counterparts. The meeting was part of a series Mr Benn is having as chairman of the EEC Council of Energy Ministers.

The ministers agreed that an early decision was needed on the question of increasing the uranium enrichment capacity of Urenco, the consortium established by the three countries which is facing problems because of Dutch concern about Germany's controversial nuclear deal with Brazil. The ministers also tacitly confirmed that the European Community fusion project, JET, was far from dead by agreeing that an early decision on its siting was needed.

No official comment was available from the UK Department of Energy on an appeal last week from the EEC Energy and Research Commissioner, Guido Brunner, who urged Britain to drop its opposition to a Euratom loans plan and its insistence on a minimum safeguard price for oil. These issues could become politically connected with the JET issue.

Brunner also appealed to the USA and Canada to maintain nuclear fuel supplies to the Community, and detailed the Community's efforts in reactor safety and nuclear export controls. A Community team of experts is shortly to visit Washington to discuss these matters.

UK mercury report

"Reassuring" is the word coming from the UK Department of the Environment (DoE) on the environmental impact of mercury in Britain. A report released last week (*Environmental Mercury and Man*, HMSO, £1.40) is the department's tenth pollution paper, and the product of an interdepartmental working group on heavy metals.

The average person in the UK, it says, is "at no discernible risk" from exposure to mercury, but for environmental and health reasons a comprehensive knowledge of the concentration and distribution of mercury must be developed and the toxicology of mercury understood. The report says more research is needed on both the conversion of mercury to and from methylmercury compounds and the environmental pathways of mercury across the water-sediment interface in the aquatic environment; research on the mechanisms of its toxic action and on low level exposure to it over long periods is also necessary.

Help for computer industry

UK government plans to concentrate on electronics as part of its drive to boost exports were revealed last week. The proposal, part of the Labour government's overall 'industrial strategy', followed an announcement that the National Enterprise Board (NEB) is to become involved in the computer industry through a newly-created NEB subsidiary, INSAC Data Systems. The idea initially is to stem fragmentation in the software sector, and later to become involved in minicomputers and peripherals. The news coincides with the publication of details of a consultant's report on the European computer industry which forecasts a bleak outlook for microelectronics in the absence of a radical restructuring of the industry.

New genetics draft

The consultative document drawn up by the UK Health and Safety Commission (HSC) last August containing draft regulations for the control of genetic manipulation experiments in Britain is thought to be still under discussion with the principal scientific interests concerned.

A report appeared last week stating that the HSC's controversial all-embracing clause, requiring notification from any person carrying on "any activity intended to alter, or likely to alter" the genetic constitution of any microorganism, has now been rewritten. The new notification clause which, it was said, the HSC intended to put in its place, reads:

> "No person shall carry on any activity which, by using biochemical manipulation of extra-cellular nucleic acids, is intended or likely
> (a) to insert genetic information into organisms, and
> (b) to circumvent the natural barriers to such insertions, and
> (c) to propagate that information, unless notice has been given . . ."

This proposal, which went out before Christmas, was a new draft for comment, and does not necessarily represent the HSC's final proposed definition of genetic manipulation experiments.

Mediterranean discussions

Representatives from fifteen of the 18 nations surrounding the Mediterranean met last week in Split, Yugoslavia, to discuss proposals for combating pollution of the sea they all share. They meet again in Athens this week.

Agriculture 1977

THOMAS H. JUKES

THE production of food is bound to overshadow many other human activities increasingly in years to come. Scientific agriculture is currently beset with many problems and would-be interveners. A trend in recent years has been to take away the authority of agriculturists over the methods used in agricultural production. The excuse seems to be that those who till the soil cannot be trusted either to 'protect the environment' or to produce food that is sufficiently free from 'contamination' to satisfy the ideals of consumerists.

An inevitable result of these bureaucratic machinations is an increase in the cost of producing food. The advent of a new administration in Washington has given rise to gleeful anticipation of more power by those who oppose many of the technological practices used in agriculture. Such opposition will increase the cost of food production further.

The success of scientific agriculture has made groceries so cheap and plentiful in the USA that the food supply is taken for granted. This may be attributable to the fact that food is so easily obtained that it has lost its aura of being a treasured gift of beneficent Mother Nature. The urban population, now two generations removed from farm life, knows very little about food production. As a result, consumerists, defined as individuals who are quite certain of what is best for the interests of consumerists, hold sway increasingly over consumers, who are apparently somewhat bewildered by it all.

For example, the successful campaign to cast out Red Dye No. 4 from maraschino cherries was based upon the finding that the dye produced tumours in laboratory rodents, and a grateful populace should have thrown the deadly preserved fruit into the garbage. However, we learn that in the supermarkets there was a run on red maraschino cherries by hoarders who feared an impending ban.

Consumerists seem not to realise or acknowledge the basic facts of agriculture and nutrition. Some of these are that food contains substances that are toxic at high levels but are sufficiently harmless in the amounts actually present that they offer no finite hazard to health. Such substances include pesticide residues as well as other adventitious contaminants. Food can be produced without such residues, or it may in some instances be refined to remove them, as in the case of sugar.

But such finickiness, carried out to quieten the apprehensions of an opulent minority, do not fit the pattern of making an effort towards achieving abundance and low cost of food in the face of a rapidly-increasing requirement by a burgeoning global population. A few months ago, there was an uproar by consumerists over the FDA tolerances for 'filth' in foods. This was a great source of amusement to anyone who had worked on a farm, and who knew, as a fact of life, that grain and rats are constant companions. Indeed, some accident of design has made rat faeces the size and shape of wheat kernels, and hence the two items are virtually inseparable by the usual screening processes. Canned vegetables and tomatoes have always contained insect parts, more so in pre-pesticide days.

A misapprehension in some quarters is that food of plant origin is somehow 'purer', 'closer to Mother Earth', and 'more healthy' than food derived from animal products. Actually, toxicants, including oestrogens, goitrogens, alkaloids, and fungal carcinogens, occur naturally far more often in plant foods than in animals. Domestic animals, serving as sources of meat, milk and eggs, can serve as 'filters' to metabolise and remove such substances, or to reduce their levels in the human diet. Cereal grains, of course, will continue to be the prime source of calories for human beings throughout the world. The business of feeding people must go on apace, and, to this end, let us hope that the 'Washington consumerist crowd' will not be given the opportunity to pour any more gravel in the gears that they introduced in 1976.

correspondence

Nuclear pros and cons

SIR,—As one of the large group of Nobel prize winners having a strong aversion to nuclear energy and its consequence but no "technological background in fields relevant to nuclear technology," I would like to express agreement with a portion of the article by Jan M. Döderlein[1] entitled "Nuclear power, public interest and the professional," but strong disagreement with much of the rest.

His point about uninformed sponsors of statements was also emphasised in 1973 by the late Eugene Rabinowitch, a person concerning whose judgment and expertise in the area of science and public policy no one could quibble: "If, at a certain point, their conclusions begin to be affected by extrascientific reasons, they must have sufficient intellectual honesty to state: 'Up to this point, I spoke as a scientist; from here on I will speak also as a politically, ethically or ideologically committed citizen . . .' " Still, anyone but the most naive petition signer must understand that sponsors of socio-scientific news releases are, and always will be, selected on the basis of the fact that the general public tends to be influenced by lists of the names of 'dignitaries' of one kind or another (perhaps I should add "so far").

One particular paragraph in Dr Döderlein's essay seemed to me to be especially noteworthy:

> Probably no technological decision in the history of mankind has been the subject of so many detailed studies, so much open discussion and such broad public participation. Amongst it all, some nuclear critics have given prominence to ethical aspects of the nuclear decision. This is justifiable to a certain extent since the application of nuclear power—indeed of any power—raises ethical questions. To carry this view to extremes in the way it is sometimes done, however, represents a vain attempt to set aside fundamental laws of nature and society.

One of the hitherto inevitable consequences of the "laws of nature and society" has been war. Given enough time, conviction and willing hands, and enough spears or rocks, the human species could certainly be doggedly annihilated. However, the existence of nuclear weapons, and the proliferating capacity to make more, makes ultimate mass slaughter not only possible but probable—I know of no technical fixes for human madness and misjudgment. Surely this statement requires no knowledge of the technology of nuclear power. The ethical and social consequences of the impending plutonium economy are at the very core of the problem[2,3]. We cannot rely, in a world of easy overkill, on "political decisions made in the face of uncertainties". The "uncertainties" associated with nuclear power, both of the electric and military variety, are unfortunately of much larger potential danger than those we have faced in our previous history.

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[1] Döderlein, J. M., *Nature*, 264, 202 (1976).

[2] *Scientists' Declaration on Nuclear Power*, issued by Union of Concerned Scientists (1208 Massachusetts Avenue, Cambridge, Mass.02138; August 1976).

[3] *The Plutonium Economy: A Statement of Concern*, issued by National Council of Churches of Christ in the USA (475 Riverside Drive, New York, New York10027; September 1975).

Evolution and growth

SIR,—One can agree with Professor Tuzo Wilson (January 20, page 196) that, on the Earth, economic growth cannot continue for ever. But can we plan for stability? It is just not true to say that "undisturbed Nature is stable". Evolution has proceeded as a result of instability. Ecosystems approach stability when they are in a steady state, but even in the steady state phase they are not in fact stable, but oscillating between quantifiable limits. Sudden increases in the size of both populations and of the area they occupy are characteristics of species in an active state of evolution; these are phases of multiplication during which selection pressure is reduced. They alternate with phases of division, in which population size and area of colonisation shrink, and during which selection pressure is increased. As a result, the phyletic line splits, isolation occurs, and new species may arise. Perhaps humanity will be moving into such a phase in the immediate future—but not necessarily so. For evolution in Man is not only controlled by biological processes. Civilisations evolve as a result of psycho-social controls. Under such conditions natural selection no longer operates within a gene-pool, but within an "idea-pool" and it is competition between ideas that leads to evolutionary progress.

Wilson may well be right when he claims that it is the exploitation of energy that has resulted in the sudden increase in the size of the human population. Certainly we can argue that traditional energy supplies must become scarcer in the future. But psycho-social evolution does not necessarily proceed in phase with biological evolution. If our technological advance continues, it may well solve the energy problem. However, it must do more than that if there is to be any further growth of population. We must also solve the problem of living space and the problem of material resources.

On the Earth there is a limit to growth imposed by the fact that both living space and resources are finite. No such limits are imposed in outer space. The only possibility of continued growth would seem to depend on space colonisation. G. K. O'Neill's space colonies (*Physics Today*, September 1974) could in their first generation exploit solar energy and lunar and asteroidal materials. They could overcome all the constraints that prevent growth on Earth.

So maybe the future of Man will lead to a bifurcation. Some of us will go into space and multiply; the rest of us will stay on the Earth, and divide.

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Journal abbreviations

SIR,—The abbreviation of periodical titles is a chore to authors and editors, and their secretaries. Abbreviations are also a source of confusion to readers, whom one hopes outnumber the authors. The saving in type-setting which is achieved by the use of abbreviations probably does not justify all this effort.

The British Standards Institution has published a word-abbreviation list (BS 4148: Part 2: 1975) but, like the *World List of Scientific Periodicals*, this does not always provide unique or unambiguous abbreviations. The French *Industrie minérale*, for example, shares the abbreviation *Ind min* with the British *Industrial minerals; J math phys* stands equally for *Journal of mathematical physics* or *Journal of mathematics and physics*.

I suggest that the practice of abbreviating titles should be discontinued, in favour of full and accurate citations.

Yours faithfully,

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news and views

Mars: questions and answers from Viking

from Geoffrey Eglinton and James R. Maxwell

THE Viking mission has been a remarkable success. So much so that NASA recently announced the start of an extended mission whereby the four spacecraft will continue to gather data on and around Mars for the next one and a half years to see out a complete Martian year of 687 Sols, each of 24.6 hours. But what of the all-important question: "Is there life on Mars?" NASA's official view of the results of the life detection experiments is that ". . . they have been puzzling. They neither prove nor disprove the existence of life on Mars". Indeed, the soil is surprisingly "active" but the question is whether this activity is chemical, biological, or possibly both. In fact, most of the researchers involved claim that none of the responses observed necessitates a biological explanation. Appropriately reactive species can be invoked in terms of a surface chemistry controlled by interaction with the atmosphere and with ultraviolet light from the Sun. Thus, understanding the properties of the Martian soils seems to be the province of physicists, chemists and geologists rather than biologists.

It is difficult not to wax lyrical about the Viking mission, with so many 'firsts' for planetary exploration. The mission has simultaneously supported four sophisticated interactive spacecraft at an interplanetary distance. The landing sites were chosen by teams only after study of radar surveys made from Earth and of detailed photography from orbit. In effect, the teams have used the orbiters as their eyes and have directed them to inspect likely sites.

The two spacecraft arrived at Mars last summer. Viking I went into orbit on June 20 and released its lander on July 20, while Viking II arrived on August 7 and sent in its lander on September 3. Pictures and data continued to flood into the Jet Propulsion Laboratory at Pasadena, California until late November when Mars passed out of effective radio contact behind the Sun. The main findings to date have been published in NASA SP-408 and in three issues of *Science* (August 27, October 1 and December 17, 1976). On December 16 the orbiters were signalled to resume photo coverage and the landers to start up their operations once again. The 18-month extended mission will provide surface and orbital photographic coverage, temperature and water vapour observations of the surface and atmosphere, seismic monitoring and additional analyses of soil and rock, including further life detection experiments. The landers may experience the onset of a planet-wide dust storm when Mars nears perihelion this spring. As part of the new phase of exploration, Viking orbiter II's orbit was changed on December 20 to give a closer approach of 800 km and a higher inclination orbit of 80°. As winter approaches in the northern hemisphere, the north polar cap should grow and the south polar cap recede, while the orbiters keep a detailed watch on changes in surface features, temperatures and water vapour concentrations. Some time this month, Viking I's orbiter will skim by the Martian moon, Phobos, and radio back pictures and thermal measurements taken from only 48 km (30 miles) distance. Rocks less than a metre across should be visible and an explanation found for the mysterious lineations apparent in the photographs transmitted last September. It is likely that the lineations—layers, flow lines, impact or volcanic crater chains . . . ? —hold the key to the origin of this small moon and its partner, Deimos.

The biological experiments are continuing on both landers with fresh soil being tested in each case. These experiments seem unlikely, however, to do little more than harden up the picture already obtained, that is, rapid release of oxygen after contact of the soil with water vapour, absorption and evolution of carbon dioxide, the oxidation of organic nutrients to carbon dioxide, and the synthesis of small amounts of organic matter from carbon monoxide/dioxide mixtures. These positive results contrast with the molecular analysis of the surface dust by pyrolysis-gas chromatography-mass spectrometry which show that it is virtually free of all pyrolysable carbon compounds. For example, no nitriles, which are pyrolytic products of amino acids were observed at detection limits of ~ 1 part in 10^9. One possibility that has been suggested is that there is a minute population of microorganisms not detected by the pyrolysis technique and which is unresponsive to the biological tests. It is more likely that the 'biology' can be treated in terms of photochemical weathering and surface chemistry.

The basic need now is to know more about the geochemical circumstances at the surface of Mars. These are clearly very different from those on Earth and a valid interpretation of results from life detection experiments needs an understanding of this chemical environment and of the processes involved in the synthesis and destruction of organic material at the Martian surface. The considerable quantities of carbon which must have been present in the early Martian surface and atmosphere and have arrived in meteorites remain to be accounted for. Certainly some is in the atmosphere and, possibly, mixed in the polar caps as CO_2 and a little CO; some carbonates may also be present in the surface materials. Presumably the processes of surface weathering, which occur in an environment dominated by ultraviolet, low temperatures and the absence of liquid water, are such that any organic compounds formed are rapidly oxidised. These processes would also erase any organic geochemical evidence of past life.

The recent paper by Oyama in *Nature* (**265**, 110; 1977) highlights the problems facing chemists and biologists. To explain the evolution of oxygen and the evolution and absorption of carbon dioxide in the Gas Exchange Experiment, Oyama proposes that nascent oxygen from ultraviolet irradiation of CO_2 diffuses to react with alkali and alkaline earth metal oxides, there, by generating peroxides and superoxides, which would be capable of evolving oxygen in the presence of water vapour. Alkaline sites would then be produced which could absorb carbon dioxide. Evolution of carbon dioxide is explained in terms of the presence of "coats" of sulphuric acid on mineral surfaces. However this scheme seems tortuous and premature when so little is known about the composition of the surface. Other hypotheses are appearing but this open-ended situation necessitates patient and detailed dovetailing of the Viking data with results drawn from laboratory simulation studies.

All of these considerations emphasise the tantalising nature of the Viking mission. The experiments on board are sophisticated in that they have revealed the complexity of the Martian surface–atmosphere relationship but they do not allow the more detailed studies which are now necessary to unravel the surface chemistry involved. Here a returned sample mission, as with the Apollo project, contrasts with the Viking type of remote analysis mission. In the case of the returned sample, new data lead to fresh hypotheses and models which can then be tested interactively, thus generating fresh data and fresh questions. With the Viking mission, the amount of testing that can be done to check on hypotheses or models is limited, though remarkable.

Fitting Flixborough into a pattern

from Henry Chilver

THE explosion of a chemical processing plant at Flixborough on June 1, 1974 was one of the most serious industrial disasters of recent times in this country. More than two years after the disaster, it is very interesting to read D. E. Newland's fascinating account (*Proc. R. Soc. Lond.* **A351,** 525; 1976) of the mechanics of the temporary pipework, the failure of which was the cause of the disaster. Catastrophes such as Flixborough raise important questions: are engineering skills adequate to build safe, complex engineering plant? What controls are introduced to ensure safe designs? Questions of these sorts raise complex issues, involving the safety of people and the supervision of engineering design and construction. All engineering disasters must be explored as thoroughly as possible and—it is hoped—understood. Each disaster has its own detailed explanation, in the case of Flixborough an explanation in terms of the structural mechanics of the temporary pipework. But how does the Flixborough disaster, seen as the failure of a vital structural component, fit into the general pattern of failures in engineering structures?

Before we attempt to answer this question it is important to try to identify some general pattern of engineering failures as a whole. A general pattern is certainly discernible in the field of construction engineering, where failures of large structures in particular are very spectacular. The first point to emphasise is that major disasters are of course rare events. This is a tribute to the quality of design and construction skills, which are generally of a high order; nevertheless, it is still important to learn, through the analysis of disaster, how we can further improve design, construction and operation.

Structural failures are of three main types, and may involve mixes of these:

(1) Unknown structural or material deficiencies; for example, the material used may be sub-standard or a vital component may be accidentally damaged.

(2) Unknown forces in the environment of the structure; for example, a long-span bridge may suffer exceptionally high winds, or a building may suffer unusually severe earthquakes.

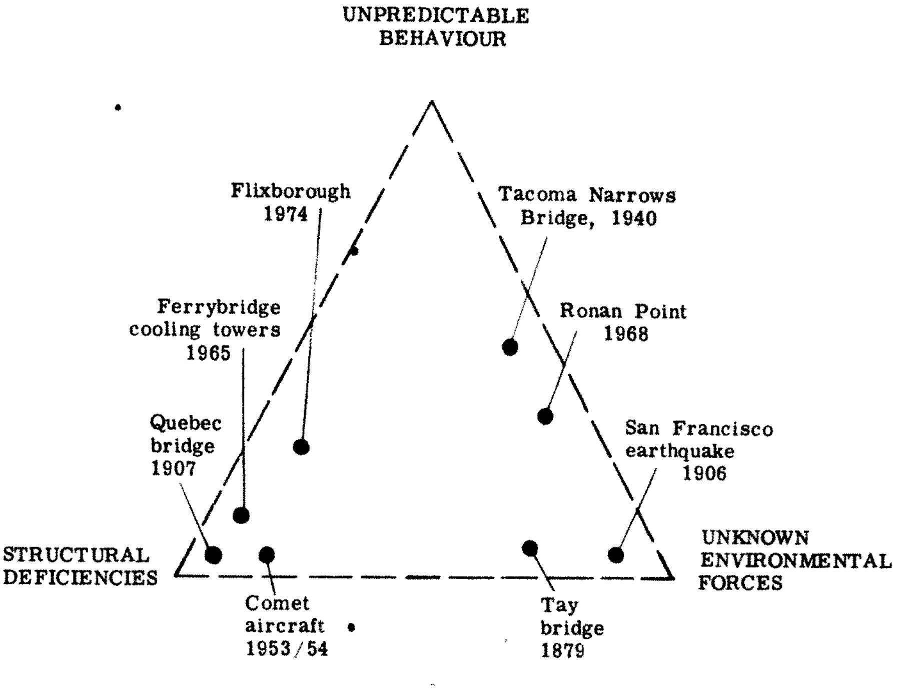
(3) Unknown forms of behaviour of the structure within its environment; for example, a new form of oscillation or buckling may occur for a new structural form.

Within the framework of these three main types of failure (and these concepts can be extended to other areas of engineering) we can construct a 'triangle' of failures, the corners of the triangle being the three main forms. Within this triangle we can locate failures of different sorts; where a failure involves 'mixes' of different types we can indicate the position appropriately within the triangle.

One of the early disasters of structural engineering was the collapse of the Tay railway bridge in 1879 (Sir Alfred Pugsley, *The safety of structures*, Arnold, 1966; this very interesting book gives more details of some of the failures described briefly in this note). In very high winds, this multi-span bridge across the Tay collapsed while a mail train was crossing; 75 people lost their lives. Although there may have been some design faults, the predominant cause was a lack of knowledge of the intensity of the actual wind forces on such a structure. This early disaster lies, therefore, towards the corner of "unknown environmental forces".

The San Francisco earthquake of 1906 is famous for many reasons, not least for the advances made, after this experience, in the design of structures against earthquake forces. But structural failures during the earthquake were predominantly due again to a lack of understanding of earthquake forces.

Taking again an example from the field of bridges, a famous accident was the Quebec bridge disaster of 1907, when a large cantilever bridge collapsed while under construction into the St Lawrence River with the loss of 75 workmen. This was caused by defec-



tive design of a member of the frame of the bridge. Good design would have shown this to be defective, and hence a basic structural deficiency was built into the structure. This disaster has a position towards the corner of "structural deficiencies".

In 1940, the suspension bridge, newly-built to span the Tacoma Narrows, near Seattle, in the US State of Washington, collapsed after violent oscillations of its deck and towers, luckily with no loss of life. Surprisingly, the failure occurred in what appeared to be only a moderate wind of about 40 mph. The cause of collapse was an inadequate understanding of the interaction of the structure with its environment, leading to large oscillations of the bridge. The resulting behaviour was unpredictable at the time, but became better understood subsequently, after research studies.

In 1953 and 1954, three accidents occurred to Comet civil airliners—all involving serious loss of life. These were caused by structural failure of the pressure cabin brought about by fatigue. The root of the trouble appeared to be cracking near one or other of the windows of the pressurised cabin. In this sense, the structure again had an in-built structural deficiency.

In 1965, a group of cooling towers at the electricity generating station at Ferrybridge in Yorkshire was blown down in a moderate gale, although they had apparently been designed to withstand high wind pressures. This failure is probably in the class of structural deficiency because the thin shells used had severe vertical cracking which probably lowered their stability.

In 1968, some multi-storey flats collapsed at Ronan Point in London after what appeared to be an explosion on one floor. The cause of collapse was traced to the explosion of leaking gas on one storey; the explosion caused structural failure on that floor which in turn led to further structural failures on other floors, rather like the collapse of a row of dominos. Although the gas explosion was unpredictable, there is a case for designing structures in such a way that collapse of one structural component does not lead to general catastrophic failure. This example lies predominantly near the corner of "unknown environmental forces".

How does Flixborough fit into this general pattern? The cause of collapse, as Newland now explains with fascinating detail and exactness, was due to the squirming and jack-knifing of some temporary pipework. The cause of failure was an inbuilt structural deficiency, not implied in the original design, in the form of the temporary pipework.

Can we make any general deductions about structural failures in the light of this very simple analysis? We begin by looking more generally at the three corners of the 'triangle' of failure. In any engineering design and construction, not all the environmental forces likely to be encountered are known, although designers hope to take account of all the relevant forces. These forces can only be found from experience and research. We must always accept some risk (although we hope this is very small) of encountering a hitherto unknown environmental force of serious magnitude. As design and construction progress, we may have to accept some unfortunate disasters towards the corner of 'unknown environmental forces' in the triangle. At the corner of 'structural deficiences' it should be possible to eliminate most disasters by thorough supervision of design and construction, particularly if conventional materials are being used. Where, as in the top corner of the triangle, an 'unpredictable behaviour' emerges, it is difficult to blame engineers for failures. In this area engineers have the responsibility, clearly, of ensuring that research is encouraged in directions in which more knowledge of behaviour is needed, but the risk of a hitherto unknown form of failure emerging should not be discounted.

The risk of structural failure is, in reality, very low. Failures frequently make headline news, and correctly so if lives are lost or put at risk. As engineering design and construction progress, so our experience of structures, of their environments and of their behaviour also evolves. We can only progress by combining experience with research into relevant problems. This is a field in which the scientific study of the natural phenomena involved has a vital role. ☐

Early assemblage of Carboniferous amphibians

from Andrew Milner

For those who are interested in the evolution of terrestriality and the early radiation of terrestrial vertebrates in the Palaeozoic, the fossil record remains frustrating. Although amphibians were present and diversifying in the Upper Devonian, it is not until well into the Upper Carboniferous that amphibians and reptiles are comprehensively represented by rich and diverse assemblages.

The five stages of the European Carboniferous are the Tournaisian, Viséan, Namurian, Westphalian and Stephanian and the majority of Carboniferous tetrapods known to us are of Westphalian age. The major Westphalian assemblages are the lake assemblage from Newsham, Northumberland, the pond assemblages from Linton in Ohio, Nýřany in Czechoslovakia and Jarrow in Eire, and the terrestrial assemblages from Joggins and Florence in Nova Scotia where the terrestrial tetrapods are found as small individuals trapped inside the hollow stumps of giant lycopods. The amphibians and reptiles from these and other contemporary localities are currently referred to about 30 families, most of which have no ancestors or close sister-groups among the known earlier tetrapods. Our concept of the coal-swamp fauna is based on the Westphalian tetrapod fauna which is of great inherent interest but is too late to be directly relevant to the fish–amphibian transition or the earliest adaptive radiation of tetrapods.

The tetrapod faunas of the Tournaisian, Viséan and Namurian are represented by a small number of specimens or restricted lake assemblages from West Virginia, Nova Scotia, Scotland and Germany. These are being restudied by several workers, in particular Panchen, Carroll, Andrews and Beaumont. Not merely are the early Carboniferous amphibians few in number but they also tell us little about the origin of later groups. Panchen (*Palaeontology* **16,** 179; 1973) has shown that *Crassigyrinus* from the Scottish Viséan, is an ichthyostegid-like relict. More recently he has studied *Pholidogaster* and *Otocratia* and found them to be colosteids with a referred specimen being an anthracosaur skull (Panchen *Phil. Trans. R. Soc.* **B269,** 581; 1975). The Viséan amphibians from West Virginia described by Romer and Hotton have proved to be all colosteids or anthracosaurs. The other major groups of this age, the loxommatids and adelogyrinids are currently being redescribed by Beaumont and Andrews respectively. But though the loxommatids, colosteids and anthracosaurs may each have a few known descendants, mostly confamilial with them, neither they nor such contemporaries as they have, shed light on the origin of most later tetrapods.

As a result of the work described above, the situation has been developing where all the early Carboniferous material which could be studied, was being studied, without any significant new material being discovered. The Namurian gephyrostegid from the Ruhr described by Boy and Bandel (*Paläontographica* **A145,** 39; 1973) was a significant find in a new area but, being a terrestrial erratic in a marine horizon, it is unlikely to be the precursor of further discoveries.

This then, is the significance of the discovery of a Namurian assemblage of fish and amphibians from Cowdenbeath announced by Andrews *et al.* in this issue of *Nature* (page 529). It has already produced as many specimens of amphibian as were previously known from the early Carboniferous of Europe and most of the bone-bed has yet to be prepared. It is still difficult to assess the diversity of the fauna for though some skeletal material is articulated, most is fragmentary and will take time to identify. So far, it appears to be a more extensive version of the Viséan assemblage from the Gilmerton Ironstone. If it is like other large assemblages from the Carboniferous, a carefull search may reveal the small and rare components of the fauna such as the occasional terrestrial reptiles now known as accidental occurrences from Linton and Nýřany (Carroll & Baird *Bull. Mus. Comp. Zool. Harv.* **143,** 5; 1972; Reisz *J. Paleont.* **49,** 522; 1975). The significance of the find is threefold. First, it offers the possibility of finding earlier, more primitive representatives of the many families of tetrapods which currently appear so suddenly in the Westphalian. Second, it will permit new morphological studies of these early tetrapods. The preservation is good and the specimens are not severely crushed. Specimens such as the nearly complete anthracosaur will yield much information. Third, the assemblage may provide some palaeoecological information about these animals. Most Carboniferous amphibian material was collected in the last century from restricted exposures and is now only available in museum collections, having been through the filter of morphologically orientated collectors. One cannot be certain what was collected and what was missed. This new find was systematically collected by gridded squares and offers the possibility of a quantitative ecological study of a Carboniferous lake fauna.

The Cowdenbeath fauna may only take us back one step from the Westphalian, but it is a long awaited step. However the most relevant amphibians to the water–land transition remain the Upper Devonian ichthyostegids from East Greenland. Although they were collected nearly fifty years ago, little progress can be reported in our understanding of them as much of their anatomy remains undescribed and no first-hand study of them has been published for twenty-five years. ☐

Bleak outlook for superheavy nuclei

from P. E. Hodgson

THE announcement by Gentry *et al.* (*Phys. Rev. Lett.* **37,** 11; 1976) of the detection in the mineral monazite of the superheavy nucleus with charge 126, and possibly of other superheavy nuclei as well, has stimulated a series of experiments to confirm this startling discovery, and also many theoretical calculations of the stability of such nuclei. Several of these have been published very recently: the experiments provide no evidence for superheavy nuclei, and the calculations make it very unlikely that they are stable enough to be observed.

The first experiment, by Bosch *et al.* in Germany (*Phys. Rev. Lett.* **37,** 1515; 1976) is very similar to that of Gentry *et al.* They irradiated small grains of monazite with 2 MeV protons, and examined the spectrum of the emitted X rays. They found no sign of a peak in the region where one would be expected if the superheavy nuclei had been present in the same proportion as claimed for the sample studied by Gentry *et al.* They go on to suggest that the peak observed by Gentry *et al.* is really due to the gamma decay of the first excited state of ^{140}Pr, produced in their sample by protons on traces of ^{140}Ce. This contaminant peak should have been removed along with the background due to other contaminants, but Bosch *et al.* claim that the background subtraction was not done correctly.

The second experiment used quite a different technique. Stephan and his colleagues from France and Poland (*Phys. Rev. Lett.* **37,** 1534; 1976) have made a mass spectrographic analysis of about one gram of a sample of monazite from the same place as the original sample. After the mass separation, the ions were collected on a quartz plate, and this was subsequently irradiated for one week in a nuclear reactor. Superheavy nuclei are expected to undergo fission when hit by a neutron, and this is very likely in the high neutron intensities to be found there. The fission fragments would make tracks in the quartz plate which would be visible under the microscope after etching. Careful examination of the quartz plate showed no fission tracks in the region where they would be expected from superheavy nuclei.

The original measurements of Gentry *et al.* were made on small inclusions of monazite in mica at the centre of the so called 'giant haloes'. The quantity of superheavy nuclei that they claimed to have found corresponds to about 10^{-4} g of monazite. The mass spectrographic analysis sets an upper limit to the amount of superheavy nuclei in monazite of 10^{-12} g per g. Thus to maintain the original result one would have to say that the concentration of superheavy nuclei in the giant halo inclusions is about 10^8 that of normal monazite, which seems very unlikely.

C. Holbrow of the California Institute of Technology has now studied haloes in detail and finds that some of the naturally-occurring haloes could be produced by ^{244}Pu (this issue of *Nature*, page 504). Although there is very little of this isotope remaining now because of its relatively short half life of 82.8 Myr, there could have been enough of it present when the mineral was originally formed for it to make haloes before it decayed. Giant haloes could also be produced by the alpha particles that are sometimes emitted during the spontaneous fission of ^{244}Pu. The X rays observed after proton bombardment of the inclusions could also come from Te fission fragments and not from superheavy nuclei. This work therefore provides an alternative explanation of the giant haloes that does not involve superheavy nuclei. Confirmation of this possibility requires mass spectrographic identification of the very small amounts of ^{244}Pu that still remain, or of its decay product ^{236}U, or of the fission fragments of ^{244}Pu.

The theoretical calculations of the stability of the superheavy nuclei of $Z=126$ are mainly concerned with the half lives for fission and for alpha and beta decay. For the nuclei to be detectable on naturally-occurring minerals the shortest of these half lives must be comparable to the estimated age of the Solar System, or about 10^8 years. If any of the half lives were shorter, the nuclei would have all decayed long ago.

Some very thorough calculations have recently been published by Möller and Nix (*Phys. Rev. Lett.* **37,** 1461; 1976) following earlier calculations by Wong (*Phys. Rev. Lett.* **37,** 644; 1976). The first calculated the single-particle energies in a spherical one-body potential and found that there is a well-marked neutron magic number at 228, but no proton magic number at 126. There is proton shell closure at 124, so on this evidence alone the nucleus with $N=228$, $Z=124$ is the best candidate.

The more detailed calculations of stability were made using the droplet model of Myers and Swiatecki (*Ann. Phys.* **84,** 186; 1974) with microscopic corrections evaluated by the Strutinsky method (Brack *et al. Rev. mod. Phys.*

44, 320; 1972). Essentially this method gives a soundly-based theoretical expression for the nuclear binding energies, and the fine adjustment of its parameters is made so that it gives the correct results for the heaviest known nuclei.

Brack *et al.* find that the nucleus with $Z=126$ and $N=228$ is stable against beta decay but has a half life against spontaneous fission of only 39 ms and an alpha-decay half life of only 18 yr. These half lives are extremely sensitive to the calculated binding energies, so that there are uncertainties in these figures of several powers of ten, but even allowing for this it seems that the nucleus is far too unstable to have survived. Major changes in the theories used in these calculations would be required to give sufficiently long half lives.

It thus seems at present that the evidence for superheavy nuclei has been seriously weakened. □

Viroids are covalently closed circular RNA

from Roger Hull

VIROIDS are one of the enigmas of plant virology. They are pathogens of higher plants and appear to exist only as self-replicating uncoated ribonucleic acid molecules with many unusual properties.

Much of the knowledge of these properties is based on studies on two viroids, the causal agents of potato spindle tuber (PSTV) and citrus exocortis (CEV) diseases. Using gel electrophoresis and electron microscopy, T. O. Diener and coworkers reported the molecular weight of PSTV to be about 80,000–90,000 (*Virology* **53**, 70, 359; 1973); J. S. Semancik and coworkers determined a molecular weight of 125,000 for CEV (*Virology* **53**, 448; 1973). These molecular weights are much smaller than those of the RNAs of plant viruses. Thermal melting curves, resistance to nucleases and gel electrophoretic behaviour show that viroids are compact highly structured molecules with a considerable amount of base pairing which makes it difficult to obtain reliable molecular weight estimates using gel electrophoresis. Several models for the molecular structure have been proposed, the most recent suggesting that they are single stranded RNA, base-paired to form hairpin-like shapes (*Virology* **55**, 70; 1973; *Virology* **63**, 160; 1975).

In a recent paper (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3852; 1976) H. L. Sänger *et al.* describe the results of experiments which lead to a different interpretation of the structure of viroids. They confirmed that viroids were single-stranded molecules by showing that native undenatured nucleic acids had similar molecular weights to denatured molecules. Using sedimentation equilibrium analysis they obtained molecular weight values of 127,000 for PSTV, 119,000 for CEV (from two different hosts) and 110,000 for the viroid causing cucumber pale fruit disease. This confirms the previous estimates of the small size of viroid RNA and shows that they have genetic information to code for a protein no larger than 10,000 daltons. In fact protein synthesis experiments (*Virology* **61**, 486; 1974) indicate that viroid RNA is not translated *in vitro*.

Sänger *et al.* went on to determine the structure of viroids. Thermal denaturation showed that each of the viroids they studied had considerable base pairing and that there was one relatively long double helical region and not short hairpin sections as in tRNA. Electron microscopy of native nucleic acid revealed that all three viroids have rod-shaped or dumb-bell shaped molecules, as had been previously reported for PSTV (*Virology* **55**, 70; 1973). When viroids which had been treated under strongly denaturing conditions were examined in the electron microscope a variety of structures ranging from rod-like molecules (renatured during the preparation process) to single-stranded circles were found. Sänger and his colleagues then attempted to analyse the nucleotides at the 5′-end and 3′-ends of the viroid molecules. Using standard techniques (phosphorylation of the 5′-end with 5′-polynucleotide kinase and ^{3}H-labelling of oxidised 3′-end and capped 5′-end by reduction with ^{3}H-borohydride) they were unable to detect either 3′- or 5′-nucleotides. From these and other observations (resistance of viroids to snake venom phosphodiesterase) they concluded that viroids are single-stranded, covalently closed circular RNA molecules.

So far in nature, circular molecules have been found for double-stranded and single-stranded DNA and have been suggested for some double-stranded RNA replicative intermediates (for example, *Virology* **70**, 360; 1976). Viroids therefore appear to be the first examples of a new class of molecule. Sänger *et al.* point out that they differ from other naturally occurring covalently closed circular molecules in that they are much smaller and that the base pairing is not between complementary strands but within one strand. This structure would also explain why viroid RNA is not translated using *in vitro* systems.

The observations of Sänger *et al* should help in the current investigations in several laboratories into the origin, mechanisms of replication and pathogenicity of viroids. One major question is whether this new class of molecule occurs normally in uninfected plants; if so, how do they become pathogenic? It is already thought that DNA might be involved at some stage in viroid replication (*Virology* **64**, 106; 1975; *Nature* **256**, 753; 1975); in fact there are sequence homologies between PSTV RNA and DNA from both infected and healthy plants (*Proc. natn. Acad. Sci. U.S.A.* **73**, 2453; 1975). The search is now on to find circular single-stranded RNA and DNA molecules of viroid size in both infected and healthy plants. I feel that the search should not be restricted to plants. It will be interesting to know how widespread is the occurrence of single-stranded circular RNA molecules and whether they are involved in diseases of organisms other than higher plants. □

Most leptons come in pairs

from W. T. Toner

THERE was a precursor to the discovery of the ψ: muons and electrons were found in such large numbers in proton–nucleus collisions that a new interaction or particle had to be invoked to account for them. (Boymond *et al. Phys. Rev. Lett.* **33**, 112; 1974; Appel *et al. ibid*, 722; *News and Views* **254**, 103; 1975). Many new studies were begun whose results continue to accumulate. Much of the data can be understood in terms of known processes once the ψ (and perhaps a small amount of charm) is included, but not all.

The large effect is small in any absolute sense, being of the order of one part in ten thousand of the production of the strongly interacting particles whose decays constitute the main experimental background to be subtracted.

The most illuminating results concern the production of pairs of oppositely charged muons or electrons. Several groups find that a substantial fraction of the leptons does come in pairs (see for example Kleberg *et al. Phys. Rev. Lett.* **37**, 1451; 1976) and that the muons are unpolarised as would be the case for electromagnetic production (Leipuner *et al. Phys. Rev. Lett.* **36**, 1011; 1976), although a Russian group has reported a large negative polarisation which is difficult to understand.

The Chicago–Princeton group (K. J. Anderson *et al. Phys. Rev. Lett.* **37**, 799 and 803; 1976; Branson *et al.* to be published) have measured muon pairs and single muons in the same apparatus so that systematic effects in any comparison are much reduced. Readers who watched *The Key to the Universe* may remember seeing this spectrometer in action in an experiment by H. L. Anderson *et al.* (*Phys. Rev. Lett.* **36,** 1422; 1976) who built it around the 184-inch diameter magnet taken from the old Chicago cyclotron. Branson *et al.* find that 0.7 ± 0.2 of all the muons they observe come from pairs. This is near enough all of them that it will not be easy to decide whether any true single muon production takes place.

The mass spectrum of pairs from this and other experiments shows peaks for the known vector mesons (ρ, ω, φ, ψ and ψ') superimposed on a continuum which drops steeply as mass increases. Earlier suggestions of a new particle with a mass of 6 GeV have not been confirmed (Hom *et al. Phys. Rev. Lett.* **36,** 1236; *ibid,* **37,** 1374; 1976). It is striking how many of the pairs have masses less than the ρ or ω mesons. These are most likely due to three-body decay modes of the eta. The effect of three body decay modes of π^0 and η can be seen even more strikingly in the results of Guy *et al.* (to be published) who studied electron production in a track sensitive hydrogen target within a neon–hydrogen bubble chamber at low (4 GeV) collision energy. They find no evidence of anomalous production. (A search at 0.8 GeV/c showed no anomaly down to the level of parts in a million (Browman *et al. Phys. Rev. Lett.* **37,** 246; 1976)).

All low energy or low transverse momentum experiments using electrons must deal with the low mass background and it takes little imagination to realise how easy it would be to underestimate the subtraction. Nevertheless we must wait for a more direct confrontation before dismissing the signals at 10 to 24 GeV of Beier *et al.* (*Phys. Rev. Lett.* **37,** 117; 1976) and at high energy and low transverse momentum of Baum *et al.* (*Phys. Lett.* **60B,** 485; 1976), or accepting the need for Lederman and White's provocative mystery particle (*Phys. Rev. Lett.* **35,** 1543; 1975).

The smooth continuum (minus decay background) is of great interest in its own right. It is thought to be due to the annihilation of a quark in the projectile with an antiquark in the target (or *vice versa*) to give a highly virtual photon which promptly materialises as a lepton pair (Drell & Yan *Phys. Rev. Lett.* **25,** 316; 1970). The theory is very far from being clear cut but is in quite surprising agreement with the overall magnitude of the effect. As this is one of the few places where there is a direct link between electron–positron annihilation, deep inelastic scattering and the collisions of strongly interacting particles, it will obviously repay further study. □

High pressure methods

from John Walker

The diamond anvil high pressure cell and its calibration by a ruby crystal are currently revolutionising the study of materials under high pressure. For the first time they have enabled pressures similar to those at the centre of the Earth to be generated and measured simply and conveniently in the laboratory, thus opening up a new realm of study.

Pressure can cause dramatic changes in physical properties such as structure, electrical conductivity and transparency. The difficulty has been that high pressures could be generated only by massive (and relatively expensive) apparatus, so experiments had to be constructed around the high pressure cell. This effectively ruled out many experiments such as those involving superconductivity, where the material has to be cooled to very low temperatures. The diamond anvil cell on the other hand is compact (15 cm long) and portable and can easily be incorporated into other apparatus. Another advantage over its ponderous predecessors is its transparency to electromagnetic radiation, which makes optical and X-ray experiments much easier.

The cell was invented at the United States National Bureau of Standards by Weir *et al.* (*J. Res. natn. Bur. Stand.* **A63,** 55; 1959). It basically consists of a pair of brilliant-cut diamonds pressed together, with the material under study in between (Fig. 1). A relatively small force applied to the large faces of the diamonds results in a large pressure between the small faces. The gasket helps to retain the specimen under study, reduces the risk of shattering the diamonds, and keeps the pressure equal and isotropic throughout the sample volume.

The diamond anvil cell thus made pressure generation much more convenient. But for some time there remained the problem of measuring the pressure exerted. Previously, one way was to measure the sudden change in resistivity of materials due to pressure-induced transitions into the metallic phase, but only certain fixed points on the scale are available. Another way is to measure the change in lattice parameter of sodium chloride crystals. This technique, however, is very slow—up to two weeks to measure a single pressure—and can only be used to measure pressures less than 291 kbar because of a phase change in NaCl at this pressure (1 bar = 1 atmosphere).

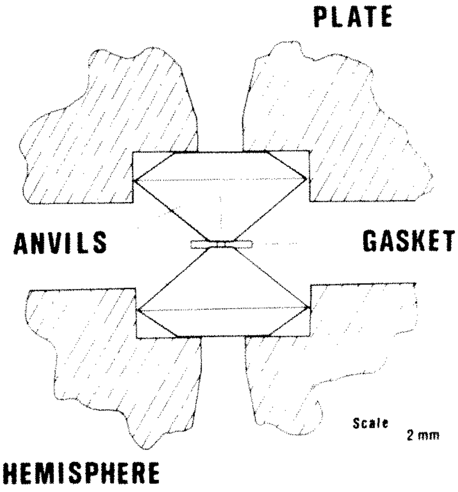


Fig. 1

In 1972 however, NBS solved the problem of pressure measurement (Forman *et al.*, *Science*, **176,** 284; 1972). The system can be calibrated by measuring the shift in the R_1 ruby fluorescence line (the ruby laser line) which is linear with pressure up to at least 300 kbars (Piermarini and Block, *Rev. sci. Instr.*, **46,** 973; 1975). The fluorescence is strong and easily excited, and the shift rate is quite large (0.036 nm kbar^{-1}). This technique has also led to a downward revision of the previously accepted pressure scale.

What are the fields of study that have benefited from these advances? In one study at NBS (Block and Piermarini, *Physics Today,* **29,** 44; 1976) the semiconductor-to-metal transition in silicon and gallium phosphide was observed by noting the pressure at which transparent crystals within the cell became opaque. A television camera enabled visual observations to be extended into the infrared. Simultaneous measurements of electrical resistance confirmed an order-of-magnitude conductivity change when opacity occurred.

The small size of the diamond anvil cell has great advantages in low temperature physics. One incorporated into a dilution refrigerator can apply pressures of 100 kbars at temperatures as low as 0.03 K (Webb *et al.*, *Rev. sci. Instr.*, **47,** 59; 1976), and has been used to study the way the superconducting transition temperature changes with pressure.

At the other extreme of temperature the transition of graphite to diamond has been studied at 260 kbars and 3,000 K (Ming and Bassett, *Rev. sci. Instr.*, **45,** 1115; 1974). Applications have also been reported in radioactivity, crystallography, optical spectroscopy and in the breakdown of lubricating fluids under high pressures (as found in bearing contacts).

The diamond anvil cell can easily equal pressures inside the Earth's crust (tens of kilobars). Hence geologists find the cell useful in experimental studies. Sung (*Rev. sci. Instr.*, **47**, 1343; 1976) has used the apparatus to study the kinetics of the olivine to spinel transition. Even pressures at the centre of the earth (3.6 Mbar) may soon be routinely available. Mao and Bell (*Science*, **191**, 851; 1976) have already broken the 1 Mbar barrier.

The technology of pressure generation and measurement has up to now lagged behind that of temperature, its complementary variable; the diamond anvil cell should help it to catch up. Every laboratory should have one. □

The sequence is not enough

from N. J. Proudfoot

An EMBO Workshop on Genes and Spacers was held at Davos, Switzerland on 10–17 January, 1977. It was organised by M. L. Birnstiel and S. G. Clarkson (Institut für Molekularbiologie II, Zürich, Switzerland).

THE aim of this meeting was clearly outlined by one of its organisers, M. Birnstiel (Institut für Molekularbiologie II, Zürich). In simple terms his message was that we had perhaps heard enough about eukaryotic gene isolation and that it was high time we actually compared data from structural or functional studies made on purified genes.

Two important contributions followed that described new DNA sequencing techniques which greatly speed up that once arduous process. First, W. Gilbert (Harvard University) briefly described the now well known, although as yet unpublished, Maxam and Gilbert chemical cleavage DNA sequencing method. By labelling the end of a DNA fragment (usually obtained by restriction enzyme digestion of DNA) with ^{32}P and then breaking it at base-specific sites using various chemical tricks, the partially degraded DNA fragments could be ordered on a gel and the DNA sequence established. Second, N. Brown from F. Sanger's laboratory (MRC Laboratory of Molecular Biology, Cambridge, UK) described the plus and minus gel sequencing method originally published by Sanger and Coulson (*J. molec. Biol.* **94**, 441; 1975). This method starts by actually synthesising ^{32}P-DNA in a test tube using *E. coli* DNA polymerase. Then with various combinations of dCTP, dTTP, dGTP and dATP (the four substrates used by DNA polymerase) the ^{32}P-DNA can be made to terminate at base-specific sites. Again, a DNA sequence may be deduced by fractionating the different DNAs on a gel. These two DNA sequencing methods have essentially increased the speed of DNA sequence analysis from the old rate of about 100 nucleotides a year to 100 nucleotides in a few 'good' days. Brown went on to show the essentially complete sequence of the bacteriophage ΦX174, 5,375 nucleotides in length at the last count. Happily for Sanger's group, their massive sequencing effort has been rewarded by the discovery of two overlapping gene systems, in which two proteins are read off the same DNA sequence, but in two different phases. That genes *E* and *D* of ΦX overlap has recently been published by Barrell, Air and Hutchison (*Nature* **264**, 34; 1976). Brown reported that two further genes *A* and *B* also overlap, so that two examples of this bizarre phenomenon occur in the same bacteriophage. Of course it remains an open and exciting question as to whether or not overlapping genes are present in other prokaryotic or eukaryotic organisms.

Two of the first eukaryotic genes to be isolated, frog 5S and sea urchin histone genes, both repeated many times over in tandem, are now well on the way to being completely sequenced. D. Brown (Carnegie Institution, Baltimore) gave an elegant report on the former system. After describing electron microscopy and restriction enzyme data on this DNA, he presented the complete sequence of the spacer in 5S DNA, showing very nicely the satellite 15-nucleotide repeat sequence originally discovered by Brownlee, Cartwright and Brown (*J. molec. Biol.* **89**, 703; 1974) and demonstrating how variation in the number of these repeats accounted for the variation in the absolute length of these genes. He went on to describe the sequence of the apparent promoter for 5S RNA, just beyond the spacer region, but apart from a repeated sequence of 10 nucleotides, no clues to its identity were observable. Several other possible eukaryotic promoter sequences, in yeast rDNA and SV40 DNA, were described by W. Gilbert and W. Fiers (Gent). Already it is becoming painfully obvious that no promoter-specific homologous sequences are generally present in eukaryotes. Histone genes from two different species of sea urchin were described by M. Birnstiel and L. Kedes (Stanford University). Long stretches of sequence from both coding sequences and spacers were presented, but even after computation (described by H. Smith (Johns Hopkins University, Baltimore)) little sense could be made. One of the great hopes for the histone gene system is that control signals before and after each of the six coding or mRNA sequences (present in each histone gene repeat unit) may be recognisably similar and that these control signals may be conserved between different species. But the data is as yet too fragmentary to be sure whether or not such hopes are justified. One such control signal, however, does appear to be generally present in eukaryotes. Proudfoot and Brownlee (*Nature* **263**, 211; 1976) recently described a sequence A–A–U–A–A–A presented in six different eukaryotic mRNAs close to their 3′-terminal poly(A) sequences. Fiers described the same sequence close to the poly(A) of EMC viral RNA and M. Billeter (Institut für Molekularbiologie, Zürich) suggested that this sequence was near the poly(A) of the related RNA tumour viruses RSV and AMV.

J. Rubin (Stanford University) presented some intriguing results on a gene from *Drosphila* of unfortunately unknown function. He described how the DNA sequences on either side of the transcribed positions of this gene appear to be nearly identical for as many as 500 nucleotides, on the basis of hybridisation data using restriction fragments and an ingenious heteroduplex experiment. More understandable were the interesting results reported by Gilbert on RSV. He described the sequence of complementary DNA made using the RSV RNA template with its natural tRNA primer and reverse transcriptase. This DNA, about 100 nucleotides long, is complementary to the 5′-end of the viral 30S RNA genome. For a long time the question has been, how is the rest of the virus copied into DNA, an essential requirement for viral integration into the host's genome. Gilbert's answer is simple. The sequence at the 3′-end of the virus contains a region of 19 nucleotides identical to the 5′-end sequence. Thus the tRNA primer may generate a second DNA primer allowing reverse transcriptase to copy the whole viral RNA.

With a few notable exceptions, the main lesson learnt from the extensive sequence data described at Davos is that knowing the sequence is not enough. Major breakthroughs must be made before any understanding of the relationship of sequence to function is achieved.

I will finish this selective account by describing two such possible breakthroughs. First, Gilbert described the use of various chemical reagents that modify DNA to locate the exact binding position of a particular protein on DNA. His model system was as usual the *lac* operon, *lac* repressor complex.

He showed very clearly, using a rather fearsome phosphate-specific reagent, that the *lac* repressor only binds on one side of the double helix. Second, both E. De Robertis (MRC, Cambridge) and D. Brown described experiments done in J. Gurdon's laboratory at Cambridge, which involved the injection of either frog 5S DNA or *Drosophila* histone DNA into the nuclei of frog oocytes (a seemingly impossible manipulation). Both experiments suggested that the foreign DNAs were transcribed specifically to generate in the first case normal 5S RNA and in the second histone proteins, detected by two-dimensional fractionation techniques. These exciting results suggest that frog oocytes may well provide an invaluable assay system for purified eukaryotic gene expression. ☐

Nova Sagittae 1977 and Vulpeculae 1976

from David W. Hughes

TENACITY and a truly astronomical memory are two attributes specifically required by the amateur astronomers who successfully search the sky for novae. Tenacity, because discoveries are few and far between—about 300 hours of painstaking observation being required to discover a nova. The encyclopaedic memory is also essential, the relative positions and brightness of about 10,000 stars must be known so that the interloper, the star which has suddenly and dramatically increased in brightness, can be easily recognised.

The last two novae found have been discovered by British amateur astronomers, the most recent one by John Hosty, a Huddersfield postman, on 7 January, 1977 in the constellation of Sagitta (the actual position is right ascension $19^h37^m08.18^s$, declination +18°00′58.2″, equinox 1950.0). This has been reported in *Circulars 3025/7* and *3030* of the *International Astronomical Union's Central Bureau for Astronomical Telegrams*. It was discovered at a magnitude of 7.2 (about three times less bright than stars visible to the naked eye, which have magnitudes less than 6.0) and has been getting steadily fainter since its discovery, as shown in Fig. 1. Most novae will decrease in brightness by about 3 magnitudes from their maximum value in about 40 d and then continue getting fainter at a slightly diminished rate.

The previous nova discovery was by George Alcock, a retired school master from Peterborough, his nova being in Vulpecula (right ascension $19^h27^m.04^s$, declination +20°21′43″) and was near its maximum brightness at magnitude 6.5 at discovery (*IAU Circular No. 2997*). As seen in Fig. 1 the nova became 3 magnitudes fainter than maximum in 42 d and 6 magnitudes fainter in about 68 d. Interestingly both novae are in the Milky Way and are very close to each other in the sky.

The instruments used to detect novae are relatively simple in comparison to other astronomical apparatus. Both Hosty and Alcock used binoculars, in fact Hosty used only half a pair of binoculars as the other half had been unfortunately broken at some time. The two instruments were 10×50 and 15×80 respectively, the first figure being the magnification and the second figure the objective lens diameter in millimetres. They would have fields of view of about 6° and 4° in diameter, the Hosty instrument detecting stars down to about 10th magnitude under excellent sky and seeing conditions. The field of view would contain, on average, 30 stars of 8th magnitude and just over 200 brighter than 10th. The daunting task of the nova hunter is to pick out the new star as he sweeps slowly over the 10,000 or so stars brighter than 8th magnitude that can be seen from a good observing sight at any one time.

What is the use of discovering novae? Well, apart from the sudden rise to fame for the finder, these stars are of considerable intrinsic interest, being at a fascinating stage in their evolutionary history. Novae occur just before a star finally dwindles to become a white dwarf. All the nuclear energy-producing reactions converting hydrogen and helium into higher atomic number elements have ended, and the star is getting its energy simply from the gravitational potential energy that is slowly released as it shrinks. The pre-nova star is typically of spectral type O or B, corresponding to a surface temperature of 20,000 to 40,000 K, with a radius about 0.3 solar radii and a mass between 0.2 and 2.5 solar masses. The absolute magnitude is around +4.5. On explosion the magnitude jumps by 11, equivalent to an increase in brightness of about 25,000 times. During the outburst the star loses on average 0.001 solar masses, the energy emitted in the few days of outburst being about 10^{45} ergs, corresponding to the energy emitted by the Sun over 2,000 yr. From spectral observations it can be seen that the luminous layers of the star are expanding outwards at velocities around 1,000 to 2,500 $\mathrm{km\,s^{-1}}$ in a rather non-uniform fashion, causing the superposition of absorption and emission features in the line spectra. At maximum the nova reaches an absolute magnitude of about −8.0 the expanding gas cloud having a radii of about 100 solar radii. After maximum the nova slowly fades into insignificance, back to the same magnitude as the pre-nova star. By carefully searching the Palomar Sky Survey photographic plates (*IAU Circular 2998*) the pre-nova star of nova Vulpeculae has been found and is a blue star of photographic magnitude 18.3. A search for the pre-nova of Sagittae 1977 has revealed three stars at the plate limit of about 20th photographic magnitude within $\sim 1''$ of the nova's position.

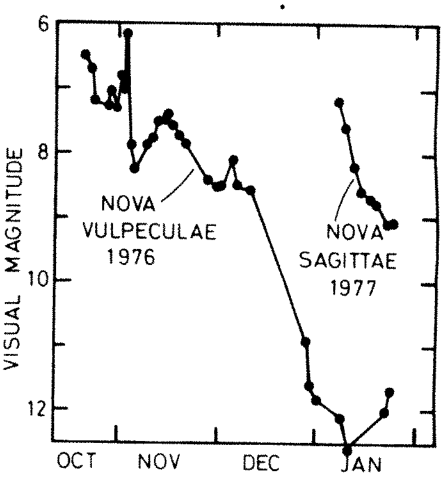


Fig. 1 The visual magnitude of the novae as a function of time. A change in magnitude by one unit is produced by a change in brightness by a factor of 2.51.

In the Galaxy there may be about 50 novae a year, only a few (2.2 yr^{-1} on average) being discovered, the rest being hidden behind dense interstellar dust clouds or being too faint at maximum.

The general excitement caused when one is discovered and the wealth of knowledge gained about this fascinating stage of stellar evolution is ample justification for the arduous and tireless search that worthy amateur astronomers like Hosty and Alcock conduct. It is very unfortunate however that so little is known about the pre-nova state of the star. So far only one spectrum, that of V603 Aquilae, has been recorded before the star became a nova. Our knowledge of what causes a star to suddenly explode in this unexpected way is thus pitifully small.

A hundred years ago

PROF. DIETERICI, of Berlin, sought to show in a public lecture, delivered a few days since, that the theories of Darwin were by no means novel, having been essentially published by learned Arabs in the tenth century.

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review article

Octopamine

Julius Axelrod & Juan M. Saavedra*

The sympathomimetic amine octopamine (p-*hydroxyphenylethanolamine*) *has the properties of a neurotransmitter in many invertebrate species. In mammals it seems to be a cotransmitter with noradrenaline.*

THE actions of neurotransmitters are ubiquitous, influencing a variety of tissues and physiological functions. For a compound to be considered a neurotransmitter, it should be present in nerves, together with its biosynthetic enzymes. When the nerves are stimulated, the transmitter should be discharged from nerve endings. Once liberated, the transmitter should be recognised by and bind to its specific receptor on the postjunctional cell membrane and produce a biological response characteristic of that cell. Mechanisms should be available to terminate rapidly the actions of the neurotransmitter. Until recently, only acetylcholine and noradrenaline were established as neurotransmitters. However, many compounds, such as dopamine, 5-hydroxytryptamine (5-HT), histamine, gamma aminobutyric acid, certain amino acids and small peptides meet many of the criteria necessary for chemical neurotransmission. Recent work has indicated that octopamine fulfils the requirements for a neurotransmitter, especially in invertebrates.

Octopamine (*p*-hydroxyphenylethanolamine) was first detected in the venom-producing posterior salivary gland of *Octopus vulgaris* by Erspamer and Boretti[1]. Soon afterwards, Armstrong and co-workers noted that relatively large amounts of the deaminated metabolite of octopamine, *p*-hydroxymandelic acid, was present in the urine of man and other mammals[2]. However, with the methods available at that time (paper chromatography) octopamine could not be detected in tissues unless a monoamine oxidase inhibitor was previously administered[3]. The injection of radioactive tyramine into rats led to the formation of radioactive octopamine which was highly localised in nerves containing noradenaline[4]. Stimulation of the splenic nerves of cats previously given ^{3}H-tyramine resulted in the release of ^{3}H-octopamine[5]. It was also observed that dopamine-β-hydroxylase, the enzyme that converts dopamine to noradrenaline[6], and highly localised in sympathetic nerves[7] also hydroxylates tyramine to octopamine *in vitro*[8]. These observations suggested that octopamine, the monohydroxylated analogue of noradrenaline, might be a neurotransmitter.

The development of a sensitive enzyme isotope method for measuring octopamine in tissues made it possible to study its normal occurrence, physiological disposition and metabolism[9]. The method depends on the ability of phenylethanolamine *N*-methyltransferase to transfer the methyl group of ^{14}C or ^{3}H-methyl-*S*-adenosyl-L-methionine to octopamine present in tissues. Phenylethanolamine *N*-methyltransferase is highly localised in the adrenal medulla and can *N*-methylate noradrenaline as well as other β-hydroxylated phenylethanolamines, including octopamine[10]. A partially purified enzyme is incubated together with aqueous tissue extracts and radioactive *S*-adenosylmethlione. The radioactive *N*-methyl octopamine (synephrine) formed is then separated from other interfering compounds by selective extraction into a non-polar solvent (mixture of toluene and isoamyl alcohol) and the radioactivity measured directly in the solvent[9] or coupled with paper chromatography of the *N*-methylated amines[11]. These methods have been found to be specific, rapid and very sensitive. As little as 20 pg can be measured using ^{3}H-methyl-*S*-adenosyl-L-methionine of high specific activity[12].

Sensitive assays using specific methyltransferase enzymes and extraction by appropriate solvents and ^{3}H-methyl-*S*-adenosyl-L-methionine have been used to measure a number of biogenic amines in the picogram range. 5-HT is measured by acetylation with liver *N*-acetyltransferase followed by *O*-methylation with pineal hydroxyindole-*O*-methyltransferase[13], an enzyme that specifically *O*-methylates *N*-acetyl-5-HT. Noradrenaline and dopamine are *O*-methylated by catechol-*O*-methyltransferase and the ^{14}C methylated amine separated by cleavage of the β-hydroxylated amine with periodate[14]. Histamine is assayed by a highly specific histamine *N*-methyltransferase from the brain[15]. An enzyme from the lung is used to methylate tryptamine[16]. Phenylethylamine[17] and tyramine[18] can be measured by hydroxylation with dopamine-β-hydroxylase followed by methylation with phenylethanolamine *N*-methyltransferase.

Octopamine in mammals

Using the isotope enzyme assay, octopamine has been found in the peripheral tissues and brain of rats and other mammals (Table 1)[19,20]. It is present in sympathetically innervated organs such as the heart, spleen and salivary gland. With a radiochemical enzymatic assay which allows the simultaneous determination of *p*-octopamine and *m*-octopamine, equal amounts of these amines were found in rat salivary glands (H. A. Robertson, A. A. Boulton, J. C. David and T. J. Danielson, personal communication). In the cat's heart, octopamine is present mainly in the atria. Octopamine is also found in the brain of all mammalian species examined, including man[12]. It is unequally distributed in the brain; highest values are present in the hypothalamus, midbrain and spinal cord. The pineal gland had the greatest levels of octopamine.

In the foetal rat brain octopamine can be detected after 15 d of gestation[21]. Up to 17 d of gestation, octopamine is the predominant amine in the foetal rat brain and is twice as high as that of noradenaline. After 18 d of gestation, there is a precipitous decline of octopamine levels in the foetal rat brain, and this coincides with a rise in monoamine oxidase. The administration of phenylalanine together with drugs that block the hydroxylation of this amino acid results in

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Table 1 Distribution of octopamine in tissues

	ng g^{-1}	References
Rat		
Heart	47	20
Salivary gland	96	19
Spleen	23	19
Pineal	450	20
Brain (adult)	3–12	11, 20, 24
Brain (foetal)	26	21
Human brain		
Hypothalamus	80	12
Temporal lobe	19	12
Caudate nucleus	11	12
Raphe nucleus	8	12
Frontal lobe	5	12
Parietal lobe	2	12
Cat heart		
Atria	100	20
Ventricle	10	20
Rabbit heart	135	20
Brain	7.5	11
Mouse heart	14	30
Brain	14	11
Goldfish heart	7	20
Octopus		
Circumoesophagal ganglia	1200	34
Superior basal lobe	4600	34
Stellatum ganglia	21	34
Posterior salivary gland	1,310,000	34
Frog heart	0	20
Lobster		
Brain	226	37
First thoracic nerve cord	830	37
Abdominal nerve cord	45	37
Peripheral nerve bundle	11	37
Earthworm		
Cerebral ganglia	7390	42
Ventral nerve	5340	42
Gut	1060	42
Muscle	233	42
Firefly lantern	125	43
Cockroach		
Abdominal cord	1010	40
Thoracic nerve cord	940	40
Brain	3950	40
Aplysia californica ganglia		
Abdominal	35	36
Cerebral	280	36
Pedal	160	36
Pleural	30	36

increases in the ratio of octopamine to noradrenaline from 2 : 1 to 12 : 1 in foetal rat brain.

Evidence that octopamine is present in nerves of peripheral tissue[19,20] and brain[22] was obtained by surgical and chemical denervation of sympathetic nerves. Denervation of the noradrenergic nerves of the rat salivary gland results in a partial disappearance of octopamine in this tissue. After the administration of 6-hydroxydopamine, a compound that destroys catecholamine-containing nerves, there is a marked reduction of octopamine in the rat heart, spleen, salivary gland and pineal gland[19,20]. An intracisternal injection of 6-hydroxydopamine causes a marked depletion of octopamine in all areas of the rat brain[22]. In the rat heart, octopamine is found in the same fraction of a sucrose density gradient as noradrenaline-containing vesicles[20]. All of these observations indicate that octopamine is highly localised in noradrenaline-containing neurons. Octopamine is present in small amounts relative to noradrenaline, yet it has a high rate of turnover in tissues, about six times that of the catecholamine[20]. Thus, the higher steady-state concentration of noradrenaline as compared to octopamine may not reflect the functional importance of each amine. Though the tissue concentration of octopamine is only a fraction of that of noradrenaline and 5-HT, the amount of the major metabolites of these amines, *p*-hydroxymandelic acid (from octopamine), 3-methoxy-4-hydroxymandelic acid (from noradrenaline and adrenaline) and 5-hydroxyindoleacetic acid (from 5-HT) are extracted in the urine of humans to the same extent[2,23]. This suggests rapid turnover of octopamine in man.

The biosynthesis and metabolism of endogenous octopamine in the rat and mouse brain has been elucidated by the administration of precursors and enzyme inhibitors[9,11,24,25]. An injection of a monoamine oxidase inhibitor causes more than fivefold elevation of octopamine in rat and mouse brain and peripheral tissues, indicating that deamination by monoamine oxidase is an important pathway for its metabolism[11,24]. A minor route of transformation *in vivo* is hydroxylation to noradrenaline[26]. The enzyme carrying out the latter reaction is localised in the liver microsomes[26]. Another minor pathway of metabolism of octopamine is *N*-methylation to synephrine by phenylethanolamine *N*-methyltransferase[10]. Tyrosine or tyramine together with a monoamine oxidase inhibitor result in a much greater increase in octopamine in rat[21] and mouse[11] brain than do monoamine oxidase inhibitors alone. In rats treated with a dopamine-β-hydroxylase inhibitor there is a considerable fall in the endogenous levels of octopamine in rat, brain, heart, and spleen[24]. An unusual metabolic route for the formation of octopamine is dehydroxylation of catecholamines[24]. The administration of L-dopa to rats leads to the increase of octopamine in the rat brain and urine of man. Dehydroxylation of catecholamines to octopamine was also demonstrated in germ-free rats, indicating that this transformation is not due to metabolism in the intestinal flora. Further evidence for dehydroxylation of catecholamines was obtained by the formation of octopamine and synephrine after an intraventricular injection of either ^{14}C-dopamine or ^{3}H-noradrenaline into the rat brain[27].

From the experiments described above, the pathway for the formation and metabolism of octopamine proceeds as follows

$$\text{tyrosine} \rightarrow \text{tyramine} \rightarrow \text{octopamine} \begin{array}{l} \nearrow \text{noradrenaline} \\ \rightarrow p\text{-hydroxymandelic acid} \\ \searrow N\text{-methyloctopamine (synephrine)} \end{array}$$

The release of octopamine from nerves was initially demonstrated by infusion of the radioactive amine into the cat spleen[5]. On electrical stimulation of splenic nerves there was a discharge of ^{3}H-octopamine into the perfusing fluid. Infusion of ^{3}H-tyramine into the spleen also resulted in the release of ^{3}H-octopamine. These findings indicate that tyramine is taken up by neurons where it is β-hydroxlyated to octopamine in the nerves. When these nerves are stimulated, the ^{3}H-octopamine is released presumably by exocytosis in the same manner as noradrenaline. Release of endogenous octopamine by nerves has been also demonstrated[22]. After stimulation of the splenic nerve of the cat there is a discharge of endogenous octopamine and noradrenaline[22]. The amount of octopamine released is only about 2–5% of that of noradrenaline. It is unlikely that octopamine and noradrenaline exist in separate neurones in the spleen and it is probable that octopamine is released from nerves as a cotransmitter together with noradrenaline. Octopamine has also been reported to coexist with noradrenaline and serotonin in vesicles of pineal adrenergic nerves[28]. After the administration of a monoamine oxidase inhibitor the amount of octopamine discharged from the cat spleen is 30–40% of the amount of noradrenaline[22]. The relatively large release of octopamine, a compound with weak pressor activity provides a mechanism for the blood pressure lowering actions of monoamine oxidase inhibitors.

Like noradrenaline, octopamine can be taken up by nerve endings of the rat brain by a temperature- and sodium-dependent process, and this process is inhibited by drugs that prevent the uptake of catecholamines[29,30]. When the brain tissue is stimulated electrically octopamine previously taken up is released. The uptake of octopamine by nerve terminals might serve as a means for rapidly terminating the actions of this amine.

In mammals, octopamine causes a transient elevation of blood pressure. The physiological actions of octopamine are similar to those of noradrenaline but it is only about 1/50 as effective[31]. Monoamine oxidase inhibitors elevate octopamine in nerves six times as rapidly as noradrenaline[20] and cause a disproportionate release of octopamine[22] and in a reduced response. Thus, octopamine because of its weaker physiological action, may modify the actions of noradrenaline.

The administration of phenylalanine and phenylalanine hydroxylase inhibitors, which results in high increases in tissue levels of octopamine[11,24], produces symptoms analogous to those of phenylketonuria. Thus, the symptoms of this disease might result from an accumulation of octopamine in the brain at the expense of noradrenaline. There is an elevation of octopamine in hepatic failure which may be associated with some of its neurological and cardiovascular pathology[32]. The levels of octopamine in the hypothalamus of children who died of acute hepatic encephalopathy (Reye's syndrome) are 12 times higher than that of age-matched controls[33]. It has been postulated that octopamine might accumulate as a false neurotransmitter and cause some of the pathology in these hepatic diseases[32].

Octopamine in invertebrates

Highest concentrations of octopamine have been found in the nervous system of octopus and other cephalopods (Table 1)[34]. The posterior salivary gland of the octopus contains as much as 1.3 mg g^{-1} octopamine. Subcellular studies showed that octopamine is localised in synaptosomes (nerve ending fractions) of the nerves[34].

Octopamine is found in relatively high amounts in single identified neurones of the *Aplysia californica* (Tables 1 and 2)[35,36]. In some nerve cells of the *Aplysia,* octopamine is present together with other putative neurotransmitters such as acetylcholine, serotonin and histamine but not dopamine or noradrenaline (Table 2). These findings indicate that in *Aplysia,* Dale's principle (one neurotransmitter for one nerve) may not hold. From these observations it is apparent that neurones containing octopamine can also exist in the absence of catecholaminergic neurones.

The nervous system of the lobster is rich in octopamine (Table 1)[19,37,38]. The amine is highly localised in the thoracic nerve cord[20] and is associated with groups of neurones along the proximal portions of the second root leaving the thoracic ganglia[38]. These octopamine-containing cells have the morphological appearance of neurosecretory cells[39] and receive synaptic inputs from the cholinergic nerves.

Relatively large amounts of octopamine are present in the central nervous system of the cockroach[40], locust[41], earthworm[42] and firefly lantern (Table 1)[43] and probably in nerves of many other invertebrates not yet examined.

Injection of ^{14}C-tyrosine into lobster nerve tissue results in the formation of radioactive octopamine and dopamine but not noradrenaline[37–39]. Dopamine-β-hydroxylase (the enzyme that converts dopamine to noradrenaline, and tyramine to octopamine), tyrosine hydroxylase and aromatic amino acid decarboxylase are also present in lobster nerves[38]. The ability of lobster nerves to synthesise dopamine and octopamine can best be explained by the occurrence of two types of nerve cells: octopaminergic cells that contain aromatic amino acid decarboxylase and dopamine-β-hydroxylase but not tyrosine hydroxylase and dopaminergic cells containing tyrosine hydroxylase and dopa decarboxylase but not dopamine-β-hydroxylase[38]. Thus nerves containing octopamine and nerves containing catecholamine seem to be spacially separated.

Octopamine-containing root cells of the lobster can synthesise ^{3}H-octopamine from ^{3}H-tyrosine[39]. When these neurones are depolarised with high concentrations of potassium there is a marked release of ^{3}H-octopamine[39]. The discharge of octopamine by potassium is highly dependent on Ca^{2+}. Thus, the octopamine-containing cells of the lobster exhibit the properties associated with the release of a neurotransmitter from nerves; liberation by depolarisation and a requirement for Ca^{2+}.

Table 2 Coexistence of octopamine and other putative neurotransmitters in single identified neurones of *Aplysia californica*

	R-2	R-14	L-11	C-1
Octopamine	2.5×10^{-6}	1.5×10^{-4}	9.1×10^{-6}	ND
5-HT	1.8×10^{-5}	3.4×10^{-5}	1.1×10^{-5}	9.4×10^{-4}
Dopamine	ND	ND	ND	ND
Noradrenaline	ND	ND	ND	ND
Acetylcholine	3.9×10^{-4}	ND	3.3×10^{-4}	ND
Histamine	3.0×10^{-6}	7.0×10^{-6}	4.5×10^{-6}	1.4×10^{-5}

Results are expressed in molarity. Molarity was calculated from volume estimation obtained by measurements of greatest and smallest cell diameters at the time of dissection (from Brownstein *et al.*[36]). ND, None detected.

Octopamine has been shown to have potent effects in insects. The local application of octopamine to the nerve cord of the cockroach causes an increase in cyclic AMP levels[44]. This, in turn, produces a stimulation of phosphorylase and a pronounced glycogenolytic effect[45]. Adenylate cyclase in cells of thoracic ganglia of the cockroach, that can be specifically stimulated by low concentrations of octopamine, has been identified[44]. Octopamine, dopamine and 5-HT are additive in stimulating adenylate cyclase activity suggesting that each of these amines activates separate receptor sites. Octopamine was found to be the most potent endogenous amine in eliciting the light-flash in the firefly lantern[46]. In locust and grasshoppers, octopamine inhibits the rhythmic contractions of muscles just as stimulation of neurones synthesising octopamine does[41,47].

The cyclic AMP levels in several lobster ganglia are elevated by octopamine which produces a characteristic pattern of nerve firing in certain ganglia cells[48]. When octopamine is perfused across the opener muscle in the walking leg of the lobster it causes a prolonged contraction[39]. This effect is stereospecific: the D(−) isomer of octopamine is 50 times more potent than the L(+) isomer.

Ionotropic application of octopamine to the nervous system of *Aplysia* revealed the presence of receptors that are very sensitive to octopamine and mediate a hyperpolarising-conductance increase in response[49]. These receptors showed a marked preference for octopamine and had an absolute requirement for a β-hydroxyl group. Cells in the cerebral ganglia are most responsive. These cells whose processes are localised in the neurophile where functional synapses are present have high concentrations of octopamine[35,36].

Incubation of the abdominal ganglia of *Aplysia* with octopamine caused a considerable elevation in cyclic AMP[50]. This increase is prevented by the adrenergic blocking agent phentolamine, and by methylsergide. Incubation of the ganglia with octopamine also resulted in an incorporation of P^{32} into a specific protein[51]. The change in phosphoprotein metabolism produced by octopamine and mediated by cyclic AMP seemed to be localised to the neurophile.

Conclusion

Octopamine is highly concentrated in neurones of several invertebrate species. Unlike in mammals, octopaminergic neurones in invertebrates are spatially separated from catecholaminergic neurons. In identified nerve cells of *Aplysia*, however, this amine coexists with other putative neurotransmitters. Octopamine is synthesised in nerves from tyrosine and tyramine and metabolised mainly by monoamine oxidase. When lobster nerves are depolarised, octopamine is liberated by a Ca^{2+}-dependent process. A specific adenylate cyclase is stimulated by octopamine in several invertebrates to activate phosphorylase in the cockroach, induce a light-flash in firefly lantern or inhibit rhythm contractions in locust muscle. All of these observations provide compelling evidence that octopamine is a neurotransmitter in invertebrates.

In mammals octopamine is localised in nerves in peripheral tissues and brain where it seems to coexist with noradrenaline, the catecholamine being present in much higher concentrations. Octopamine is released from nerves together with noradrenaline and it may under certain conditions modify the actions of the adrenergic neurotransmitter. Octopamine is present in unusually high concentrations in certain neurological and hepatic diseases and may have a pathophysiological role.

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articles

Haloes from plutonium minerals and fission alphas?

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The normal alpha decay of naturally occurring ^{244}Pu *can be expected to have produced pleochroic haloes detectable in existing rocks. Several methods of identifying such haloes are described here. Giant haloes might also be produced by the* α *particles that occasionally accompany the spontaneous fission of* ^{244}Pu. *It is plausible to speculate that in ideal circumstances such gaint haloes might be as large as 100* μm, *and that the X rays recently induced from central inclusions of giant haloes and identified as L rays of* $Z = 126$ *might instead have been K X rays from Te fission fragments.*

BECAUSE of its relatively short half life of 82.8 Myr, ^{244}Pu is present only in small amounts in the contemporary Earth. Mass spectrometry of meteorites, however, shows that at the time the Earth formed the ratio of $^{244}Pu/^{238}U$ was about 0.014 (refs 1 and 2). Consequently, for a period in the Earth's history there was sufficient ^{244}Pu present to affect the economy of fissionable materials and to form minerals. Isotopes which are products of the fission of ^{244}Pu have been identified in meteorites and the Earth[3-4], and work has begun on the mineral chemistry of plutonium[5]. It would be interesting to know the enrichments, fractionations, crystallisations and mineral formations of which plutonium is capable in geochemical processes. In the past such minerals may have been concentrated sufficiently to produce normal and anomalously large pleochroic haloes. Identification and study of these haloes might contribute to more accurate estimates of the concentration and the nature of plutonium minerals at different times in the Earth's history.

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Pleochroic haloes are spherical shells of coloration usually about 40 μm thick surrounding small grains of radioactive mineral. They commonly occur in pegmatites around grains of minerals such as sphene, apatite, zircon and monazite containing uranium and thorium. Haloes should also have formed around grains that have in the past contained sufficient amounts of plutonium minerals. Although haloes occur in several different types of rock, they have been studied mostly in biotite mica largely for reasons of experimental convenience[6–10].

Haloes generated by 10^{10} to 10^{14} atoms of ^{244}Pu would only rarely be distinguishable visually from haloes generated by ^{238}U or ^{232}Th. But plutonium haloes might be distinguishable by mass spectrometric detection of residual ^{244}Pu, its descendant ^{236}U, or by the presence of characteristic fission fragments in the central inclusions.

An interesting possibility is that some haloes around inclusions containing more than 10^{15} atoms of ^{244}Pu might be larger than uranium and thorium haloes. For every 3.2×10^5 decay α particles with energies around 5 MeV, an inclusion of ^{244}Pu will produce one α particle accompanying a spontaneous fission. More than half of these spontaneous fission α particles will have energy greater than 15 MeV and might produce shells of coloration with thicknesses as great as 100 μm.

The possible existence of plutonium haloes is interesting not only as further evidence of the presence and activity of a mineralogy of primordial ^{244}Pu in geological processes. The subject has interest because it may be possible to identify as ^{244}Pu haloes the 'giant haloes' reported by Gentry[10]. Such an identification might provide a basis for an alternative interpretation of the data from which Gentry *et al.*[11] deduced the existence of elements $Z = 116$, 124, 126 and 127—the so-called superheavy elements.

Normal haloes

Halo coloration behaves like the photographic process. There is a threshold of exposure below which no colouring occurs; there is a region in which optical density of the mica increases approximately linearly with exposure; above certain exposure levels there is saturation, and coloration remains constant. Finally, very intense exposures will produce complicated bleaching and reversal. Deutsch[7–9] studied naturally occurring uranium and thorium haloes in various biotite micas and also artificially induced coloration in these micas with α particles from radon. The coloration is triggered by sufficient ionisation density, but the mechanism is not known. They concluded that a fluence (time integrated flux) of about 10^{13} α particles per cm^2 is required to initiate coloration in the laboratory. They also found that natural fluences must be four to seven times greater than artificial ones to make the same effect in a given mica, and that different micas may differ widely in sensitivity[7–9].

Since the fluence must exceed a threshold for coloration to occur, no halo will form beyond the range of the most energetic α particle. Also, if the fluence drops below $\sim 10^{13}$ α particles per cm^2 there may not be sufficient ionisation to colour the halo. Consequently, the size of the halo is limited by the range of the α particles or by inverse square dissipation of the fluence below the threshold, whichever happens first.

Under the assumption that the fluence threshold in nature is 4×10^{13} α particles per cm^2, the minimum number of α particles that will colour a halo out to 18 μm from an inclusion small compared with the halo size is 1.6×10^9. This number of α particles would be produced by the decay of 5.4×10^8 nuclei of ^{244}Pu, and if they occupy 10% of the volume of the inclusion, 1 μm is the characteristic dimension of the smallest inclusion for which the size of its halo is determined by the range of α particles rather than by the inverse square law. In Fig. 1 such haloes correspond to inclusions with initial amounts of ^{244}Pu above the horizontal line labelled 'threshold of formation of visible haloes'.

Can such haloes be present in existing rocks? Can more than 5×10^8 atoms of plutonium have been concentrated into mineral grains 1 μm in diameter or larger? The likelihood of such an occurrence depends on the extent of geochemical concentration of plutonium at different geological times, and on the abundance of ^{244}Pu at the time of the formation of the mineral grains.

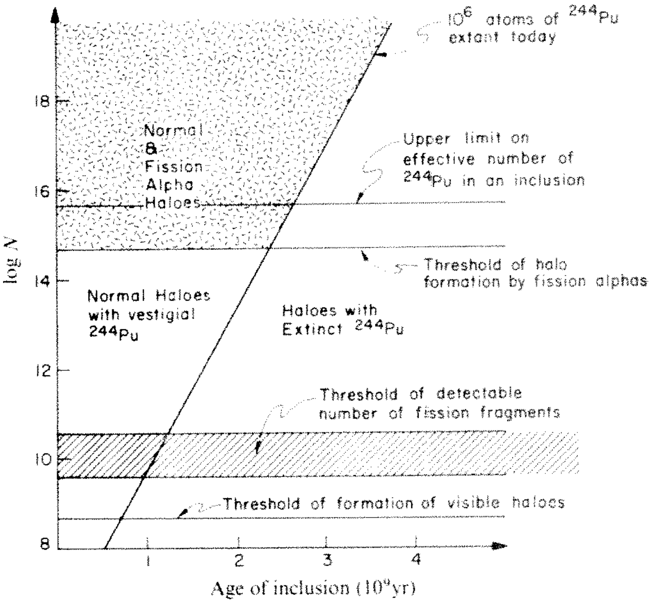

Fig. 1 Relationship of number of ^{244}Pu atoms in a mineral inclusion to the age of the inclusion. The diagonal line corresponds to 10^6 atoms of ^{244}Pu at present extant in the inclusion. The other features of the graph are discussed in the text. N is the number of ^{244}Pu atoms in inclusion at the time of formation.

Ores and minerals of most elements can be found somewhere in nature with enrichments of at least 10^6–10^8 times their average abundance in the Earth. Very pure grains of minerals and, in some cases, the elements themselves, can occur within such ores. The frequency of occurrence of the enriched ores and grains depends on the elemental abundance and the particular chemistry of the minerals. Hoffman *et al.*[12] examined some ore rich in bastnaesite, (Ce,La)FCO$_3$, from Mountain Pass, California, in which cerium is some 10^6 times more abundant than its average in the Earth. In 8.5 kg of the bastnaesite, they found 2×10^7 atoms of ^{244}Pu which they estimate is an enrichment of 2×10^8 over the average amount present in the Earth.

Moreover, bastnaesite may not be the best mineral in which to look for the maximum concentration. Considerably greater concentrations of plutonium probably occur in monazite. One may expect plutonium to concentrate approximately as thorium because the most stable oxidation state of the two chemicals is four, and in this they differ from all other lanthanides and actinides. But thorium is only 0.39% of the Mountain Pass bastnaesite[13] while it occurs as up to 28% of some monazites[14]. (Monazites from Mountain Pass are about 3% thorium.) Furthermore, monazite is a phosphate mineral, and during formation of phosphates of uranium, thorium and plutonium, the fractionation of plutonium is favoured by a factor of two to three (ref. 5).

The abundance of ^{244}Pu drops by 10^6 every 1.65×10^9 yr. Thus, pleochroic haloes will be produced by plutonium far enough back in geological history that the combination of concentrating processes and abundances suffices to produce grains 1 µm in diameter, or larger, containing 5.4×10^8 or more atoms of ^{244}Pu. Whether this occurred in rocks young enough to be part of the Earth's crust today needs to be determined experimentally.

If, when examined in thin sections, samples exhibited haloes with sharp rings and had only one radioactive element, Th, U or Pu, in the centre, they could be distinguished easily. Ideally the three α particles emitted as a ^{244}Pu nucleus decays to ^{232}Th would produce rings of coloration in biotite at about 14.2, 14.6 and 17.8 µm from the surface of the inclusion. Among the bands of colour that uranium would produce would be two at 15.3 and 19.9 µm. Two of thorium's several colour bands would occur at 12.4 and 20 µm. Unfortunately, inclusions can be expected to contain all three elements. Furthermore, the rings are not sharp except under rare ideal circumstances and are usually seen as bands or even discs of colour. Colour bands produced by plutonium will normally be difficult to distinguish from those from uranium. If no uranium were present, it might be possible to recognise a combination plutonium–thorium halo. It might be possible with such a combination to reproduce the characteristics of the X haloes mentioned by Gentry[6].

A more certain identification of plutonium haloes would be the mass spectrometric detection of vestigial ^{244}Pu, its descendant ^{236}U or fission fragments in a halo inclusion. Detection of plutonium with a mass spectrometer requires the presence in the inclusion of about 10^6 atoms. Their presence will depend upon the age of the inclusion and the original amount present. The diagonal line in Fig. 1 corresponds to inclusions containing 10^6 atoms of ^{244}Pu today. Therefore, points in the stippled region represent inclusions which should still have detectable amounts of ^{244}Pu. For example, an inclusion formed 1.4×10^9 yr ago must have contained 10^{11} atoms of ^{244}Pu in order to contain 10^6 atoms today. Points to the right of the diagonal line represent extinct plutonium haloes that would not now contain enough ^{244}Pu to distinguish them from thorium haloes.

Uranium 236 is formed in the decay chain of ^{244}Pu and has a half life of 2.4×10^7 yr. In transient equilibrium the ratio ^{236}U/^{244}Pu will be 0.41. Measurement of ^{236}U would suggest the presence of a plutonium halo.

Another distinctive feature of ^{244}Pu that might permit unambiguous identification of plutonium haloes is its relatively short half life for spontaneous fission. For ^{244}Pu the fission half life is 6.6×10^{10} yr (ref. 15), much less than the $\sim 10^{16}$ yr for ^{238}U, $\sim 10^{17}$ yr for ^{235}U and the $> 10^{21}$ yr for ^{232}Th (ref. 16). Consequently, for every 800 normal α-particle decays there will be a spontaneous fission producing two fragments.

The characteristic asymmetric mass and charge distributions of these fragments might serve to identify the halo as a plutonium halo. Of course, in such a distribution any given mass constitutes at most 7% of the total number of fragments, and if the level of detection by mass spectrometry is 10^6 atoms (about the best possible and it varies widely with the element), there will have to be a total of 10^7 fragments present to make the most abundant fragment isotopes detectable. Thus, about 4×10^9 atoms of ^{244}Pu is the smallest amount that will produce a detectable number of fission fragments. At least another factor of 10 is needed before quantitative measurements are reliable. Therefore, inclusions originally formed containing more than 4×10^{10} atoms of ^{244}Pu should contain enough remnant fission fragments to indicate the earlier presence of ^{244}Pu. The threshold for detection of fission fragments in an inclusion is indicated in Fig. 1 by the horizontal cross-hatched band.

Fission fragments have the advantage of indicating the nature of the halo whether the ^{244}Pu in the inclusion is extinct or not. Of course, the haloes may have been subject to the vagaries of physical and chemical action that remove fission fragments or in some other way vitiate their usefulness as identifiers. One of the most abundant fragments is xenon, but it is especially easily removed because it is a gas.

Anomalously large haloes

The fission of ^{244}Pu could lead to the production of haloes larger than those commonly observed. In about one out of 400 fissions an energetic α particle may be expected to be emitted[16]. In the spontaneous fission of ^{252}Cf, in the induced fission of ^{240}Pu and in all other cases of fission studied these α particles have a roughly Gaussian distribution of energies from 0 to 30 MeV with a peak around 16 MeV (refs 16–19). Because about 0.6 of the emitted α particles have energy greater than 15 MeV and a 15-MeV α particle has a range of $\sim$ 100 µm in biotite, it would seem possible to generate haloes as large as 100 µm or even larger, certainly much bigger than the normal size of 40 µm.

The production of large plutonium haloes, however, is by no means as probable as the production of normal plutonium haloes. To generate a number of fission alphas to produce a fluence of $\sim 10^{13}$ α particles per cm^2 at a distance of 100 µm from the surface of an inclusion requires an inclusion so large that self-absorption becomes a serious problem.

A simple model illustrates the difficulty. Consider a 100-µm diameter spherical inclusion of a pure crystal of 'plutonite', a hypothetical mineral of plutonium phosphate of density 7.5 g cm^{-3} and molecular weight 339. The Bragg–Kleeman rule predicts that a 15-MeV α particle will have a range of 79 µm in this mineral. Self-absorption in the inclusion will be important.

A simple numerical integration of this model taking self-absorption into account and assuming that the emitted fluence of α particles has a Gaussian energy distribution with a peak at 16.4 MeV and FWHM = 10.8 MeV, yields a fluence of 10^{13} α particles per cm^2 at 50 µm from the surface and 1.9×10^{12} α particles per cm^2 at 100 µm. Both of these numbers are smaller than the 4×10^{13} α particles per cm^2 assumed above as necessary to initiate coloration of a halo. Making the inclusion larger or smaller does not eliminate the problem.

Nevertheless, the possibility of anomalously large haloes still needs consideration chiefly because the threshold of coloration is poorly defined. Our knowledge of the threshold is based on experiments which assumed reciprocity between intensity and duration of exposure and used much higher rates of exposure than occur around haloes in nature. Yet reciprocity is known to fail in the coloration processes[6].

The possible failure of reciprocity is interesting in two respects. Unlike normal haloes which are terminated by the limits of the ranges of the alphas emitted from the inclusion, haloes produced by α particles from spontaneous fission would be limited by the inverse square law. Consequently, the volume 'outside' the halo would be subject to an appreciable α-particle fluence over periods of geological time. There have been no laboratory investigations of circumstances like these and it is conceivable that coloration might occur at fluences much below the nominal threshold.

We recognise that these inclusions and their immediate environment would be subject to strenuous conditions very different from those under which the laboratory determinations of threshold were made. The plutonium mineral would in the course of the lifetime of a halo convert itself into a thorium mineral. Also inside and around the inclusion there would be a large fluence of α particles from the normal mode of decay. The inclusion in the model described above would contain 7×10^{15} atoms of ^{244}Pu which would emit 21×10^{15} α particles with energies from 4.5 to 5.2 MeV. This is a fluence of 5×10^{20} α particles per cm^2 at 18 µm from the surface. These α particles would deposit more than 15,000 J in and around the crystal, which would consequently be metamictised, undergoing changes of density and varying degrees of recrystallisation. The amount of helium generated would be large, as can be appreciated by realising that if it did not diffuse away it would produce a pressure more than 600 atm at room temperature in the 68-µm radius volume of the inclusion and halo.

Furthermore, there would be 14×10^{15} electrons emitted from the two stages of beta decay in the normal decay chain of ^{244}Pu. With energies from 0 to 2 MeV these electrons would extend several hundred μm out from the inclusion and would deposit about 500 J in the spherical volume 150 μm in radius centred on the inclusion. Although this is about 1,000 times more energy per unit volume than is deposited by a threshold fluences of 4×10^{13} α particles per cm^2, it is unlikely that the β particles produce coloration by themselves because there is no evidence that similar intensities of β rays outside large inclusions rich in Th or U have produced coloration[6]. A large inclusion rich in ^{244}Pu, however, has a unique combination of an appreciable α particle fluence along with a large β-particle fluence. It is possible that the β particles might activate the mica and lower the coloration threshold for the α particles.

The foregoing considerations suggest that a variation in the threshold for coloration by large amounts from values based on laboratory experiments is definitely possible.

Giant haloes

There is an important reason for pressing to its limits the possibility of ^{244}Pu as a source of large haloes. Such haloes have been observed, but no adequate explanation of them exists. Figure 2 shows the relationship between distribution of haloes and their size, as observed by Gentry[10]. The sharp peak at 37–43 μm is attributed to normal uranium and thorium haloes. The peak at 50–58 μm has been attributed to the 10.6-MeV α particle from ^{212}Po* which has a branching ratio of 1 : 5,500 in the usual thorium decay chain[10]. (As can be seen, this peak appears too high for such a small branching ratio.) The larger haloes are unexplained.

The possibility of producing large haloes depends on the ratio of the partial half life T_1 for emitting long-range α particles during spontaneous fission to the overall half life T_0 of the nuclide. It is helpful to relate this ratio to our model mineral inclusion and the nominal threshold fluence φ required to produce a halo out to a distance r_0 from the surface of the assumed spherical inclusion of density ρ, molecular weight A, and radius r. A useful inequality between T_1 and T_0 follows from the fact that the ratio of the number of radioactive nuclei in the inclusion to the number of long-range α particles passing through a surface a distance r_0 from the inclusion must be greater than or equal to the ratio T_1/T_0, that is

$$(4/3\pi r^3 \rho N_a)/(A 4\pi (r + r_0)^2 \varphi) \quad R \geqslant T_1/T_0$$

where N_a is the Avogadro constant. The 'registration factor' R has been introduced to take into account enhanced ionising efficiency, absorptive losses, different concentrations of plutonium, or deviations of actual from nominal threshold. Let $a_0 = (r + r_0)/r$, then

$$T_1 a_0^2 (a_0 - 1)/R \leqslant T_0 \rho N_a r_0/(3A\varphi)$$

is an inequality which must be satisfied in order to produce giant haloes by α particles accompanying fission.

In terms of our hypothetical plutonite inclusion with $r = 50$ μm, $r_0 = 100$ μm and a threshold fluence of 4×10^{13}

$$T_1 \, 18/R \leqslant 1.1 \times 10^6 T_0$$

For plutonium $T_1/T_0 = 32 \times 10^4$, so in 'plutonite' a registration factor of 5.2 is needed to satisfy the inequality. The numerical calculation referred to above gives $R = 0.25$. Thus to meet the conditions of the inequality, the registration factor must be increased by a factor of about 20.

It is easy although not altogether convincing to increase R. The uncertainty in the threshold is probably a factor of at least 40. It is only necessary to believe that a fluence of 10^{12} α particles per cm^2 over geological time would produce colour in a region adjacent to one undergoing coloration and in the presence of a large fluence of electrons. In the absence of any information about the mechanism of colour formation in haloes, this is at least a possible assumption. In addition, the peak in the Bragg ionisation curve folded into the inverse square decrease in ionisation is, at the end of a 100-μm track, 30% higher than at the minimum on the track. With about half the nominal fluence it would be possible to achieve coloration at the ends of tracks although some intermediate parts along the tracks would not be coloured. Or again, metamictisation or recrystallisation might lower the density sufficiently to increase the range by 20% and thus the fluence at r_0 by as much as 40%. Together these factors could produce an R of 16.

It is necessary to recognise that only pure 'plutonite' has been discussed here. Most mineral inclusions are not pure. Mixtures of plutonite with other substances to lower the registration factor by factors of two, three or more are likely. This dilution of plutonium might have been offset by preferential fractionation of plutonium such as was mentioned above[5]. It is therefore appropriate to modify our model inclusion by assuming that it is only 50% plutonite. Under this assumption the registration factor for the model inclusion will be $R = 0.13$; optimally, for this model, R might equal 8.

Therefore, it seems likely there could be haloes of 50–60 μm size, and it seems conceivable that optimum conditions could result occasionally in giant haloes of up to 100 μm around an inclusion containing ^{244}Pu. Such a sequence of probabilities is consistent with the distribution of halo sizes shown in Fig. 2.

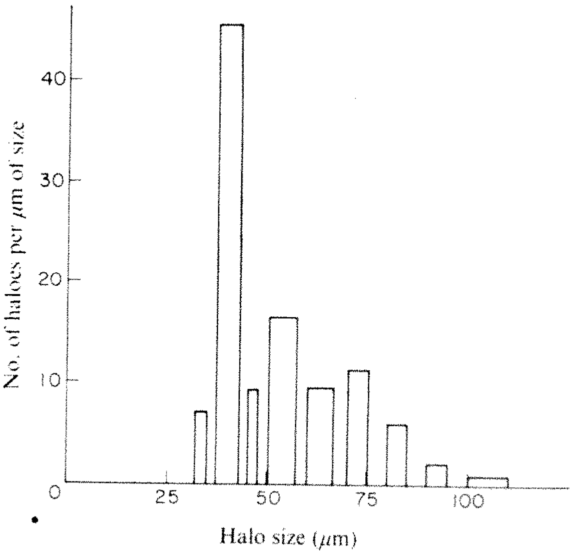

Fig. 2 Distribution of haloes by size. Size is measured from the nearest surface of the inclusion to the outer edge of the halo. These data are adapted from ref. 10.

It should be quite straightforward to determine whether a giant halo is due to ^{244}Pu. The size of the halo will be directly related to the size of the inclusion. The largest haloes should occur only around inclusions with dimensions of 50–100 μm; the smaller the inclusion, the smaller the halo. The short half-life of ^{244}Pu suggests that the substantial concentration of ^{244}Pu required to make a giant halo would have been more likely long ago, so the rock containing giant haloes should be old. Giant plutonium haloes should also have quantities of fission fragments in and around the central inclusion which could easily be detected by mass spectrometry and even by X-ray spectrometry. (Our model plutonite inclusion and halo would contain about 10^{13} fragments.) The fragments are 800 times as abundant as the α particles emitted in fission. There might also be detectable amounts of ^{244}Pu in the inclusion, but if the minimum number of nuclei needed to produce a fission α particle halo is 10^{15}, Fig. 1 shows that the rocks in which such haloes occur must be younger than 2.5×10^9 yr if plutonium is to be detectable.

Because of the vigorous thermal, physical and chemical history of the inclusion, it is likely that most etchable fission tracks in the vicinity of the inclusion would have been annealed out.

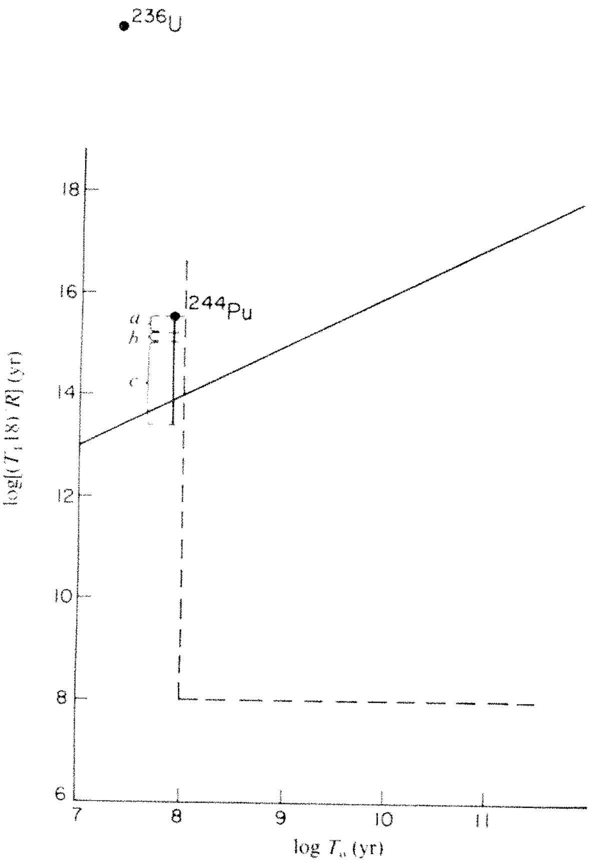

Fig. 3 Relation of α particle-fission half lives and overall half-lives that will permit production of giant haloes by fission alphas. The diagonal line is drawn assuming a threshold fluence of 4×10^{13} α particles cm^{-2}. Possible corrections to the location of ^{244}Pu are *a*, Bragg ionisation, *b*, changes in density and range, and *c*, lowering of the coloration threshold. At present no isotopes are known with values in the preferred region below the diagonal line and above and to the right of the dashed lines. ^{244}Pu is the closest.

The graph in Fig. 3 plots $T_1 a_0{}^2(a_0 - 1)/R$ against T_0. (R is a function of a_0, so a_0 cannot be varied independently of R.) For a nuclide to produce giant haloes by fission α particles, its half lives must define a point in the region below the diagonal line defined by the inequality, and above and to the right of lines corresponding to 10^8 yr. These last constraints are not well defined but take into account that if its half life were much shorter than the age of the Earth the likelihood of a nuclide being around long enough to be incorporated into a mineral is small. Plutonium 244 probably represents a lower limit.

Superheavy elements

The possibility of giant haloes from fission α particles is relevant to the suggestion of Gentry *et al.*[11] of the existence of the elements $Z = 126$, 127, 116 and 124, based on the X-ray spectra produced by proton bombardment of the inclusions of several giant haloes. The analysis of their spectra is complicated by the necessity of subtracting a large background from a nearly equal foreground. It seems possible that they may have been studying plutonium haloes and inclusions rich in fission fragments. The very elements they must argue are not contributing to the peaks attributed to superheavy elements are ones expected to be among fission fragments: Te, In, Sb and Xe. Moreover, the intensity of the peak identified as the $L_{\alpha 1}$ of $Z = 126$ is roughly of the right size and energy to be K_α X rays from $\sim 10^{11}$ Te fission fragments, somewhat less than the number to be expected in our model sample.

The association of the peak with $Z = 126$ is most strongly supported by the apparent discrepancy of 173 eV between the observed position of the peak (which is about three channels and 350 eV wide) and the expected position of the centroid of the Te K_α lines. The background, however, is so large that it seems possible that the subtraction procedure might shift the apparent position of the centroid enough to account for the difference.

The uncomfortable stretching of parameters required to form a ^{244}Pu giant halo is not the only alternative to balance against the hypothesis that the superheavy elements exist in their inclusions in quantities of "several hundred picograms" (ref. 11). There is the alternative of attributing giant haloes to an as yet undiscovered isotope or its short-lived descendants. The isotope could of course be a superheavy, but it seems as plausible that a new isotope could be a member of a known atomic species with a half life of $\sim 10^9$ yr and either a fission half life not much greater or a daughter isotope with a short fission half life.

Conclusions

It is reasonable to expect to find in nature pleochroic haloes produced by extinct or nearly extinct minerals of ^{244}Pu. These could be identified visually, by vestigial ^{244}Pu or ^{236}U in the central inclusion, or by the presence of the appropriate fission fragments.

Larger than normal haloes, perhaps up to 100 μm, might arise from the energetic alpha particles that occasionally accompany the spontaneous fission of ^{244}Pu. If the giant haloes observed in nature are not produced by the fission α particles of ^{244}Pu, they might, as Gentry *et al.*[11] suggest, come from elements $Z = 126$, 127, 116 and 124, or they may be caused by the fission α particles of some as yet undiscovered isotope.

To determine the relative probabilities of these phenomena, further study of the chemistry of plutonium pertinent to geological processes and mineral formation is required. Also, the coloration process should be examined, especially in the presence of combinations of fluxes of different radiations.

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Tomato bushy stunt virus at 5.5-Å resolution

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The coat of tomato bushy stunt virus is built from protein subunits having rigid domains connected by a flexible hinge. Two states of the hinge are present in the $T = 3$ icosahedral structure. Each subunit has a binding site for RNA on its inner surface.

DESIGN principles of regular viruses were first clarified by Caspar and Klug[1] who showed that quasi-equivalence could reconcile the variability of subunit packing (required to construct a shell of sufficient size) with the specificity of subunit bonding, necessary for self-assembly. The small, spherical RNA viruses pose two important structural questions: how is quasi-equivalent packing of identical protein subunits achieved, and how is the nucleic-acid chain accommodated in the particle? We describe here a 5.5-Å resolution electron-density map of tomato bushy stunt virus (TBSV), and derive some initial answers.

The TBSV particle comprises a single molecule of RNA (MW 1.5×10^6), 180 coat protein subunits (MW 41,000 each) and perhaps one copy of a larger polypeptide[2-4]. The coat protein forms a $T = 3$ icosahedral surface lattice, with prominent dimer clustering at the outside of the particle[5]. This design implies that a single kind of polypeptide chain packs in relation to its neighbours in three slightly different ways (Fig. 1). X-ray crystallography[6,7] has revealed the orientation of local symmetry axes and suggested that the differences between quasi-equivalent contacts is related to RNA-induced perturbations.

The structure has been solved using multiple isomorphous replacement, combined with phase refinement by icosahedral averaging. Problems for data collection due to the large unit cell were solved using a focusing X-ray camera[8], and a strategy for oscillation photography appropriate to the restriction that only one exposure can be obtained per crystal[9]. As in the 5-Å resolution study of the tobacco mosaic virus disk[10], a problem of comparable magnitude, exploitation of the non-crystallographic symmetry[11] has been important for obtaining a good map.

Data collection and phase determination

TBSV crystals, obtained as described before[7], are cubic, space group I23, $a = 383$ Å. Each lattice point of the body-centred cubic cell is occupied by one virus particle, such that the crystallographic point group is the tetrahedral subgroup of the viral icosahedral symmetry.

X-ray intensities were measured by oscillation photography from crystals of the native virus and of two derivatives, $PtCl_6^{2-}$ and Hg^{2+} (Table 1). Coordinates for the $PtCl_6^{2-}$ derivative were available from earlier work, refined in projection to 9 Å (ref. 7). A difference-Fourier *hk*0-projection for the Hg^{2+} derivative at 9 Å, (from a 6° precession photograph), using signs from $PtCl_6^{2-}$, revealed two sets of quasi-equivalent sites (six sites per icosahedral asymmetric unit). One of these corresponds to the CH_3Hg^+ sites used in the 16-Å work[7], and the other to positions near the subunit tips. After conventional refinement, the average figure-of-merit was 0.57 (Table 2). Fivefold molecular averaging was then carried out using recently developed methods[11]. After one cycle of phase refinement the mean figure-of-merit was 0.76 (Table 2). Previous experience suggested that additional cycles were unlikely to improve the map further[7,10]. A second map was computed using the combined phases, icosahedrally averaged[11].

Electron density map

It is convenient to look at the electron density map in sections normal to a crystallographic twofold (arbitrarily chosen as the *Z*-axis). The 16-Å resolution map[7] shows that the quasi-threefold axis is approximately parallel to the nearest strict twofold (Fig. 1), so that quasi-threefold related electron density features appear in almost the same *Z*-sections. The triangle shown in Fig. 1 is thus a useful choice for the icosahedral asymmetric unit, and we chose to contour on acetate sheets a region somewhat larger than this triangle, indicated by the rectangalar outline.

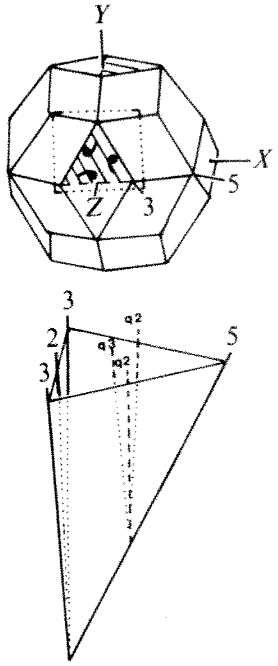

Fig. 1 Orientation of TBSV symmetry axes and choice of icosahedral asymmetric unit. *a*, Rhombic triacontahedron, showing some icosahedral symmetry axes. This figure has icosahedral symmetry, and it is useful for representing a $T = 3$ icosahedral surface lattice. The triangular half-face (shaded) is an asymmetric unit of the surface, that is, this region, replicated by the various symmetry operations, will cover the entire surface. In a $T = 3$ lattice, there is a local threefold axis near the centre of this triangle, relating the three quasi-equivalent subunits in the icosahedral asymmetric unit. Note that *X*, *Y* and *Z* are twofold axes. The rectangle indicates the area of the electron-density map, computed in sections normal to *Z*, chosen for contouring (compare Fig. 2). *b*, An asymmetric unit of volume in a region governed by icosahedral symmetry. This volume, replicated by the various symmetry operations, will fill the rhombic triacontahedron in (*a*); it can be thought of as the domain swept out by the upper shaded triangular half-face in (*a*) on a series of concentric rhombic triacontahedra of continuously increasing radius. Orientations of local (quasi) symmetry axes (denoted by small numbers) are also shown. The quasi-twofold and threefold axes around each fivefold axis intersect it, about 60 Å out from the particle centre.

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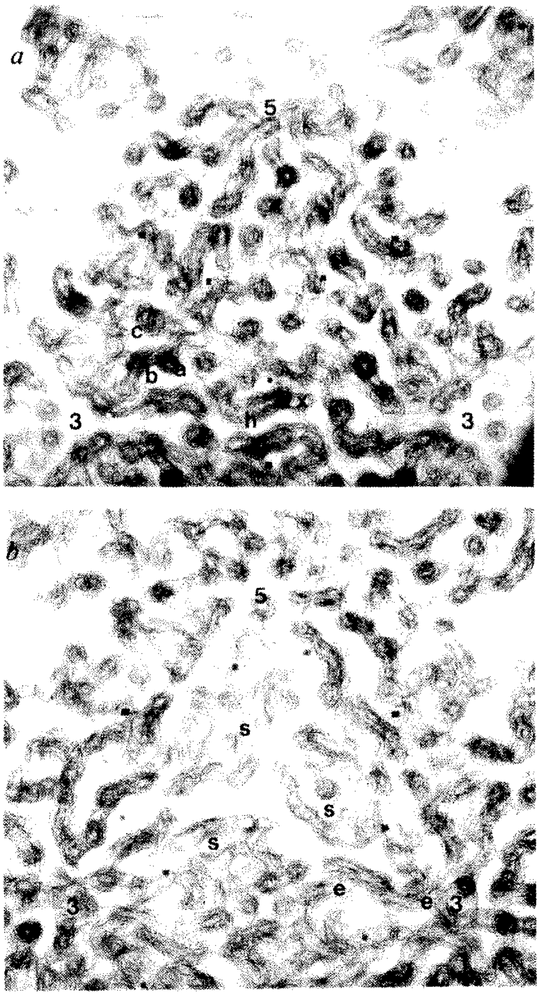


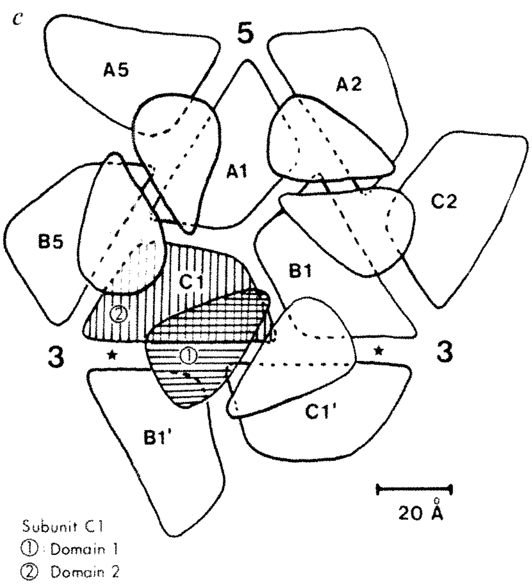


Fig. 2 Views along Z through sections of the TBSV 5.5-Å electron density map. *a*, $Z = 125$–140 Å; *b*, $Z = 110$–125 Å. Figure 1 gives orientation. Some features of secondary structure are indicated by lower-case letters: *X* is the inter-domain connection; *h*, the tangential rod; a, b and c, some radial rods; s, the saddle-like feature; and e, the extra density. The subunit boundaries may be judged by reference to (*c*). *c*, Packing of subunits and domains. A1, B1 and C1 represent subunits packed in one icosahedral asymmetric unit, related to each other by quasi-threefold rotation; A2, . . . , A5, are related to A1 by fivefold rotations; similarly, B1, . . . , B5 and C1, . . . , C5; B1′ is related to B1 by a strict dyad, A2 to B1 and B5 to A1, by quasi-dyads. The domains are indicated in rough outline: barrel-shaped domains 1 protrude outwards from the surface, making only twofold contacts; the more extensive domains 2 make contacts across all adjacent symmetry axes, forming a continuous polyhedral shell. The shading of C1 indicates which domain 1 is associated with the labelled domain 2. Domains 1 for C2, B1′ and A5 are not shown. Stars indicate the interrupted contact ('cleft'), occupied by RNA. The scale is the same for (*a*), (*b*) and (*c*).

Table 1 Statistics of data collection to 5.5 Å

	Native	$PtCl_6^{2-}$	Hg^{2+}
Total fully recorded reflections measured	69,000	75,000	65,000
No. of independent reflections measured	26,700	27,100	26,700
$R_{\text{sym}} = \sum_{h,i} \lvert I_i - \bar{I} \rvert / \sum_h \bar{I}$	0.100	0.133	0.145
Mean isomorphous difference $= \langle \lvert I_{\text{nat}} - I_{\text{der}} \rvert \rangle / \langle I_{\text{der}} \rangle$	—	0.29	0.56

Data were collected by oscillation about [101], the shortest axis in reciprocal space; rotation from 0° to 20° (where 0° corresponds to [101] coincident with the X-ray beam) includes more than 90% of the 29,000 independent reflections to 5.5 Å. The crystals thus always present a rather plate-like aspect to the X-ray beam, which is much smaller than the crystal diameter, so absorption effects are minimised. The oscillation range of a single 20–24-h exposure was 55–65′ of arc. Exposure usually exhausted the crystal lifetime, so that we could not add partially recorded reflections from abutting photographs[12]. Partial reflections, about ⅓ of those recorded, were not included in the data set but the ratios of the measured intensities of some of them to the intensities of corresponding symmetry-related whole spots on the same film, were used to obtain an accurate estimate of crystal orientation and hence an accurate discrimination between fully and partially recorded reflections[9]. Because of the small cross-fire in the focused beam and the small mosaic spread of the crystals, a 10–15′ overlap of adjacent exposures ensured that all reflections were fully recorded on film. Films were densitomered using a PDP-11/20-linked Optronics Photoscan and a modified version of the program SCAN 12 (ref. 13). The output included an integrated intensity and a standard deviation for each reflection, the latter determined from the actual standard deviation of the measured background. The three films in a pack were scaled to each other by the method of Fox and Holmes[14]. The approximately 3,000 fully-recorded reflections were sufficient to generate a Wilson-type plot of ⟨I⟩ against $\sin^2\theta/\lambda^2$, and these plots were used to determine relative scale and temperature factors for each film pack.

The electron-density features within this icosahedral asymmetric unit begin at about $Z = 112$ Å and extend out to $Z = 170$ Å. At $Z \sim 110$ Å there are faint and rather wispy but relatively continuous features. From $Z = 112$–140 Å, the volume is entirely filled with dense features, and at larger values of Z, density is concentrated more and more around the strict and quasi dyads, leaving large gaps in between. The icosahedrally-ordered part of the virus structure can thus be described as a roughly 30-Å thick, compact polyhedral shell, from which 90 protrusions, the dimer-clustered morphological units so clearly seen in electron micrographs, emerge.

Boundaries and domains

Figure 2*a* shows the composite electron density of sections from the central part of the polyhedral shell. Local threefold symmetry is visible in the central part of the asymmetric unit, and there is striking similarity of corresponding parts of quasi-equivalent subunits. Assignment of subunit boundaries was aided by the requirement that symmetry-related density features near an axis must belong to different subunits, even though they may seem weakly connected. A good illustration of the clarity of most boundaries is the gap running more or less along the lower edge of the icosahedral asymmetric unit (connecting the strict threefolds) in Fig. 2*a*. The quasi-equivalent boundaries, not as pronounced in this view, show up equally well if viewed along the local dyad.

The most outstanding characteristic of the subunit is that it falls into two very distinct, globular domains (Fig. 3). The smaller of these (domain 1) forms half of one of the dimer-clustered protrusions; the larger (domain 2) forms part of the polyhedral shell. Domain 2 has a rather distinct, saddle-like feature just against its innermost surface, which we describe in the next section. The two domains are connected at only one place by a narrow

Table 2 Phase determination

	Site	Z	B	Heavy-atom parameters x	y	z
$PtCl_6^{2-}$	A	55	53	−0.0157	0.1493	0.3060
	B	49	46	0.0756	0.0481	0.3014
	C	53	48	−0.0619	0.0203	0.3001
	D	19	35	−0.1132	0.0059	0.2933
$R_c = 0.58$, $R = 0.56$, r.m.s. f_c/r.m.s. $E = 1.86$, mean ΔF/mean $F = 0.12$						
Hg^{2+}	A1	80	25	−0.0372	0.0905	0.3339
	B1	71	27	0.0358	0.0923	0.3344
	C1	81	35	0.0010	0.0271	0.3360
	A2	71	25	−0.0680	0.1205	0.4234
	B2	66	28	0.0822	0.0976	0.4277
	C2	76	25	−0.0146	0.0145	0.4301
$R_c = 0.55$, $R = 0.53$, r.m.s. f_c/r.m.s. $E = 1.9$, mean ΔF/mean $F = 0.26$						

Z, occupancy in electrons, on experimental absolute scale[7]; B, heavy-atom 'temperature factor'; R, $\Sigma||\Delta F_{obs}|-|\Delta F_{calc}||/\Sigma|\Delta F_{obs}|$, all reflections; R_c (Cullis R factor), R for centric reflections; E, lack of closure at best phase; f_c, heavy-atom structure factor; r.m.s., root-mean square.

Statistics of molecular averaging up phase refinement.

Figures of merit: initial (DIR), 0.57; final (combined), 0.76.

Comparison of calculated and observed structure factors:

$R = \langle||F_{obs}|-|F_{calc}||\rangle/\langle|F_{obs}|\rangle = 0.37$.

Mean phase difference $= \langle|\alpha_{calc}-\alpha_{DIR}|\rangle = 47°$.

Mean phase change after combination $= \langle|\alpha_{final}-\alpha_{DIR}|\rangle = 25°$.

Alternate rounds of phasing and icosahedrally-constrained least-squares refinement[7,15] used starting coordinates obtained as described in the text. Coordinates and occupancies for both derivatives were refined in projection, first at 9 Å then at 5.5 Å, and finally in three dimensions against a set of approximately 3,500 reflections representing a random sample of resolution and intensity ranges, selected with the criterion that the precision of heavy-atom intensity differences meet a pre-determined minimum. After refinement in projection, self and cross-difference maps gave no evidence for additional sites, a conclusion confirmed in three dimensions. Site names correspond to the subunit to which they were subsequently seen to bind (compare Fig. 2*c*), and only one icosahedral asymmetric unit of sites is given. The quoted values of R_c and so on, refer to the 3,500 well-measured reflections used for the refinement. For icosahedral phase refinement, an initial map, computed on a 1.1-Å grid for accurate interpolation[11], was averaged about the icosahedral fivefold axes to determine the molecular envelope; density not part of the particle to which these axes refer is smeared out by this procedure, enhancing the boundary contrast. The final envelope for an entire virus particle is a rhombic triacontahedron (Fig. 1) with roughly cylindrical 'pillars' at each twofold and quasi-twofold position, where subunit tips protrude[7]. Averaging and setting all density outside this envelope to zero yielded a symmetrised map (2.2-Å grid), from which structure factors were computed. The agreement between observed and calculated amplitudes is tabulated: most of this residual comes from the scatter of the figure-of-merit around its mean value in resolution shells[11]. Calculated and DIR phases were combined using the Sim formula[16].

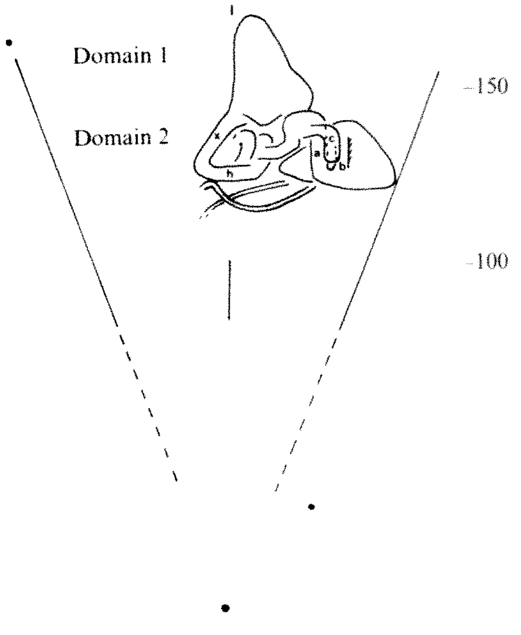
region of strong electron density: each domain thus appears to contain one terminus of the polypeptide chain.

Domain 1 is about 30 × 30 × 25 Å in overall dimensions. It consists of two approximately parallel layers, oriented such that one layer forms an extended and tight dimer contact with domain 1 of the twofold (or quasi-twofold) related subunit. The general distribution of electron density in this domain resembles very strongly the β-barrel structures, also found dimer-clustered, in immunoglobulin fragments[17,18] and in superoxide dismutase[19]. The outer layer of the barrel is markedly curved; the inner appears quite flat, especially in the contact region. The strand directions in each layer cannot be established with certainty in a 5.5-Å map, but there is definite indication of a radial rather than tangential course. The outer layer of the barrel is markedly curved; the inner seems quite flat, especially in the contact region. The strand directions in described below.

The connection of domains 1 and 2 is well defined and occurs at the point marked with a cross in Figs 2*a* and 3. Domain 2 itself is considerably more complex than domain 1, both in its internal structure and in its interactions with adjacent subunits. Nevertheless, its boundaries appear rather well determined, with one major ambiguity (see below). In tracing a boundary, we used the symmetry criteria described earlier and assumed that the domain is a reasonably compact globular unit. Figure 2*c* shows schematically the relation of domains 1 and 2 and the nearest-neighbour packing in the protein shell.

Domain 2 has a number of rodlike features indicative of α-helices (these are absent in domain 1). These rods are oriented more or less radially with one important exception, which we refer to as the tangential rod (h, in Fig. 2*a*). As seen in Figs 2*a* and 3, one end of this rod connects with the feature that forms the junction between the two domains. The tangential rod is 15 Å long (2–3 turns of α-helix) and located close to the dyad, such that the distance between twofold-related helical axes is 10 Å. Each rod is tilted slightly out of the plane normal to the dyad, and we have chosen the enantiomorph such that the tilted rods have a left-handed relationship. This packing is appropriate for right-handed α-helices[10,21]. Closely-packed, radially-oriented rods in domain 2 show the same relative tilt, and the twist of the outer wall in the domain 1 β-barrel is also consistent with this choice. The three most prominent radially-directed rods (a,b,c in Fig. 2*a*) seem to be in direct contact. Their lengths correspond to 2–3 turns. Rods a and b are tightly packed, whereas rod c is somewhat farther away. The principal ambiguity in boundary tracing, mentioned above, concerns the assignment of c and some connected density to subunit C1 (Fig. 2*c*). The alternative assignment of c to A1 leads to

Fig. 3 Schematic representation of the electron density in one subunit, viewed normal to the adjacent dyad and looking toward that dyad from the quasi-threefold. If the drawing is taken to represent C1 in Fig. 2*c*, then the direction of view is downwards in the plane of that figure. The lobe at the outside of the subunit (Z = 140–170 Å) is the β-barrel of domain 1; the cross indicates its connection to the more extensively α-helical domain 2; the 'saddle' lies beneath domain 2, and a tentative interpretation in terms of two stretches of RNA backbone is shown in the diagram.



a more extended domain 2, but the choice is arbitrary. In the pentamer (or hexamer) contact regions near the bottom of domain 2, the density has a layer-like character, suggestive of β-sheet. Rods a and b are packed partly against this sheet-like feature. There are a number of shorter pieces of rodlike density, less clearly α-helices. One of these occurs close to the local threefold axis: the three such symmetry-related rods appear to form a sort of rudimentary, left-handed, three-stranded coiled-coil[21].

We estimated the volume occupied by all the strong features of one subunit as $51{,}000 \pm 6{,}000$ Å^3, where the upper and lower limits correspond to generous and restricted estimates of surrounding side chain, Fourier cutoff and so on. If all this volume were protein, the corresponding MW would be $39{,}000 \pm 5{,}000$ (taking $\bar{v} = 0.74\ \mathrm{cm^3\ g^{-1}}$). Twelve nucleotides, our upper estimate for RNA in the saddle feature, occupy about 3,400 Å^3 (taking $\bar{v} = 0.51\ \mathrm{cm^3\ g^{-1}}$). We can thus account for most of the protein subunit, although some 10% of the chain could be outside strong density in the present map.

Nucleic acid

A significant problem in interpreting the TBSV electron-density map has been to establish criteria for recognising RNA. Strength of the density alone proved insufficient: the features described below are among the strongest in the map but no stronger than the α-helices. This is not surprising because partial occupancy of polynucleotide binding sites and other sorts of static disorder would diminish the corresponding density. A general picture of the nucleic acid might be one of ordered regions of backbone, bound to protein, interspersed with more disordered regions at smaller radii. The overall appearance of the map at about 110 Å from the particle centre is consistent with this view: strong features trail off towards the centre in little streamers of density. The distribution of this streaky density suggests that the polynucleotide chain may enter or leave a binding region in more than one way, but the strength of the beaded features to which they join indicates a definite conformation in the binding site.

A striking aspect of the map is the complicated and cross-connected texture of features between 110 and 120 Å, in contrast to the rather clear sheet-like or rod-like density of domains 1 and 2. In this region, each icosahedral asymmetric unit shows three very similar, though not precisely identical, saddle-shaped features, about 30 × 20 × 10 Å in extent (s in Fig. 2*b*). Each of these saddles forms the innermost density associated with one protein subunit. In addition, there is a set of strong features with no quasi-equivalent counterpart extending from near the strict twofold toward the strict threefold axes; we refer to this as extra density (e in Fig. 2*b*). Since the protein subunits are identical and since RNA is the only other significant component, this extra density is likely to represent part of the nucleic acid chain. Inspection of a 5.5-Å resolution map of yeast-phenylalanyl tRNA (computed as an F_{cal}, α_{calc} map by A. Jack, MRC Laboaratory of Molecular Biology, Cambridge) shows that the sugar phosphate backbone has a quite characteristic appearance: a continuous stretch of density with barely resolved maxima spaced at distances of 6–7 Å, at or near the positions of phosphates, giving it a beaded aspect. Both the extra density and certain parts of the edges of the saddles have just this 6–7-Å beading. Moreover, the saddle lying beneath subunit C is connected to the extra density at several points, with the 6–7-Å periodicity continuing from one to the other. We therefore conclude that a significant part of the density between 110 and 120 Å represents polynuceotide chain.

The saddle features form rather distinct lobes of density just against the bottom of domain 2. We followed possible RNA backbone configurations in this region, although, in view of the ambiguities involved, such a tracing is rather speculative at this resolution. We believe that we can recognise two pieces of RNA backbone (Fig. 3). One of these emerges from the particle interior and passes near the strict or quasi-twofold; the second forms the outer rim of the saddle. We detected 10–12 possible phosphates. Density between these chains could in part be nucleotide bases, and the backbones are so oriented that they could be joined by two or three base pairs in the A-RNA geometry.

No simple description would seem to summarise protein-RNA contacts. The structure of domain 2 shows that several portions of the polypeptide chain, separated in the amino acid sequence, must come together to form the shallow pocket in which the saddle feature lies. Stretches of α-helix, as well as a region that may be β-sheet, are involved.

States of the protein subunit

The clarity of the electron density map has facilitated quantitative analysis of subunit similarities. The coat protein assumes two clearly different conformational states, depending on whether it is near a local or a true dyad in the $T = 3$ surface lattice. The individual domains 1 and 2 are essentially invariant, and a hinge between the rigid domains relates the two subunit conformations.

Corresponding well defined electron density maxima could easily be recognised in all three quasi-equivalent subunits and their superposition determined after appropriate rotation and translation. Referring to Fig. 2*c*, subunits A and B superpose very well, whereas A (or B) and C do not. Taken separately, however, domains 1 and 2 of A (or B) can be superposed with their analogues in C as well as A and B can be matched to each other. This implies that the relative orientation of domains 1 and 2 in C is different from their relative orientation in A or in B, but that individual domains are nearly invariant. The hinge between domains is

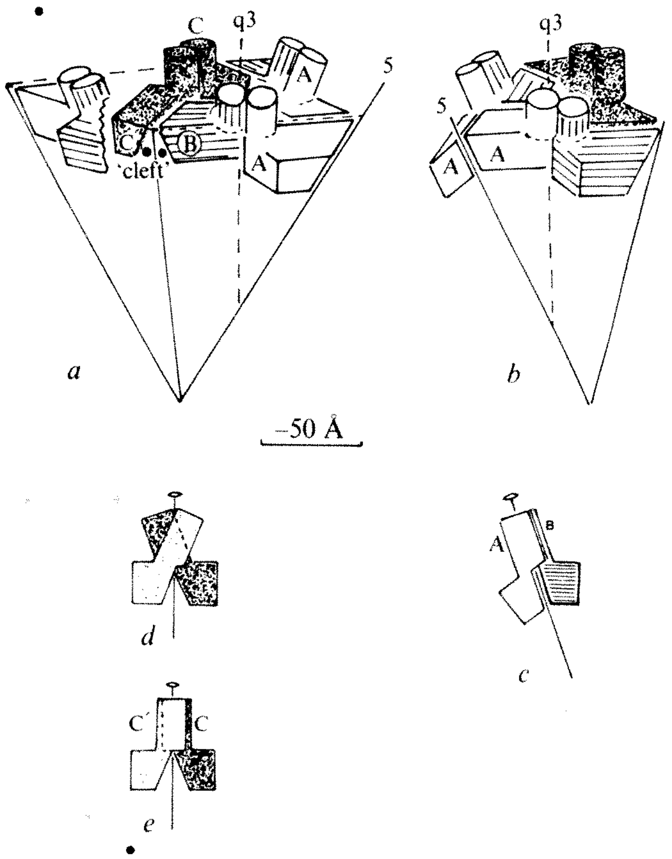


Fig. 4 Packing diagrams, showing subunits and relative orientation of the two domains. *a*, View similar to Fig. 1*b*, with a second icosahedral asymmetric unit replicated by the strict twofold axis. Each subunit is represented by a cylinder (domain 1) on a block-like base (domain 2). The three quasi-equivalent subunits are shown: white (A), hatched (B) and stippled (C). *b*, View of an icosahedral asymmetric unit, rotated 120° with respect to the view in (*a*). Considering just the domain 2 contacts, note that the A–A contact about the fivefold axis and the B–A contact across the quasi-dyad are quite tight, whereas the B–C′ and C–C′ contacts about the quasi-sixfold and strict twofold axes are opened up to produce a cleft. The difference in the angle of contact across quasi (A–B) and strict (C–C′) dyads requires a change in the angle between domains 1 and 2 to preserve the dimer pairing of domains 1. This is shown in (*c*–*e*): the A–B contact (quasi-dyad) is depicted in (*c*). If the contact between the domains 2 were altered, without a relative reorientation of domains 1 and 2 in each subunit, the C–C′ contact (strict dyad) would appear as in (*d*). In fact, there is rotation by approximately 20° about a hinge between domains 1 and 2, producing the sort of contact shown in (*e*).

located just above the end of the horizontal α-helix (Fig. 3). The hinge rotation axis is approximately parallel to the axis of the tangential rod, and the angle of rotation is about 20 (for C, relative to A or B). There are small, though significant, differences between A and B, but the hinge position is clearly the same for both.

The inter-subunit contacts of the hinged protein are represented in Figs 2*c* and 4, which show how the hinge leads to quasi-equivalent subunit bonding. Domains 1 have large and essentially identical contacts across both local and strict twofold axes. Domains 2 are likewise in extensive contact around the local threefold axis, which, for these domains, approximates well to an exact triad. Schematically, the polyhedral shell formed by all the domains 2 can be represented by 60 wedge-like trimers in continuous contact across local dyads but with only their outer edges in contact across strict dyads (Fig. 4). This produces a cleft connecting strict threefold (quasi-sixfold) axes. The quasi-sixfold relationship is thus very inexact: domains 2 of C1 and B5 in Fig. 2*c* are related essentially as A1 and A2, whereas the analogous contact between B1′ and C1 (star in Fig. 2*c*) is largely broken by the cleft. This is in fact the only place where contacts are not basically the same for all three quasi-equivalent subunits. It is the hinge between domains 2 and 1 of subunit C that permits the cleft to open (Fig. 4*e*); without it, TBSV protein could form only a simple $T = 1$ icosahedral shell. Since 'extra' electron density features appear in the cleft, but not in the tight contacts between C1 and B5 or A1 and A2, RNA may play a significant role in regulating assembly of correct-sized, $T = 3$ particles. RNA-free, $T = 1$ structures have indeed been observed in studies of reassembly of the related turnip crinkle virus[23].

Domains and hinges

The generalisation that large polypeptide chains will fold into smaller, compact domains has gained considerable strength from the several proteins solved thus far with subunit molecular weights greater than 30,000 (refs 24–26). The two domains of TBSV protein have approximate molecular weights of 15,000 and 25,000; in the scheme of Levitt and Chothia[27] they seem to be members of class II (all β) and III ($\alpha + \beta$), respectively.

The TBSV subunit is not the first example of a protein with flexibly-linked domains. The immunoglobulins are the classic case of such a structure[17], and the relation between light chains in the Mcg Bence–Jones dimer[18] is precisely analogous to the situation in TBSV: one of the chains in this dimer (the chain with a 'heavy-chain-like' configuration) bends at a hinge point, in order to conserve strong inter-molecular contacts. This type of structure could lead to controlled polymorphism in assembly.

The presence of RNA in the cleft at the C1/B1′ interface seems to dictate the nature of inter-molecular contact and hence the choice of a $T = 1$ or $T = 3$ design. Since the angle of the contact is determined by the hinge angle, binding of ligands to residues in the hinge region, or at the domain 1/domain 2 interface, could likewise influence the mode of association. In general, a subunit with flexibly-connected rigid domains can be a useful element for controlled switching between distinct states of a macro-molecular assembly. Work on a 2.8-Å resolution map of TBSV is underway.

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letters to nature

Far infrared observations of IRC + 10216

THE detection of infrared radiation in the 1.2–20 μm band from IRC + 10216 was first reported by Becklin *et al.*[1]. Toombs *et al.*[2] determined its angular size by lunar occultations at 2.2, 3.5, 4.8 and 10 μm, and deduced that the source is a late-type carbon star surrounded by a dust shell with two components: a bright, optically thick central component with a characteristic diameter and temperature of 0.4″ and 600 K, respectively, and an outer component of diameter 2″ and a temperature around 375 K. The longest-wavelength ground-based observations were made by Low, Rieke and Armstrong[3] at 34 μm, and the only other far infrared measurements are those of Campbell *et al.*[4]. We present here measurements of IRC + 10216 at 100 μm made with the 91-cm telescope of the NASA G. P. Kuiper Airborne Infrared Observatory[5].

The infrared photometer consists of a gallium-doped germanium (Ge:Ga) photoconductive detector with a Mosfet pre-amplifier and load resistor, all of which were cooled to liquid helium temperature. Fabry optics were used to reduce the background radiation on the detector, and a 4-mm aperture was used to define a 0.9′ circular field of view. The instrumental passband was defined by cold filters (crystal quartz and Yoshinaga filters) at the field aperture. The spectral response of the detector–filter combination shown in Fig. 1*a*, was measured by using a Perkin–Elmer grating spectrometer and normalised to a Golay detector.

The Bent Cassegrain mode was used with the infrared photometer mounted in the aircraft cabin. Modulation and background calculations were achieved with an oscillating secondary mirror operated at 30 and 100 Hz. The half-power beamwidth was 0.9′ and the separation between modulated beams was 2′.

Owing to mechanical difficulties with the aircraft, only one flight was made on March 9, 1976. Mars was observed for calibration

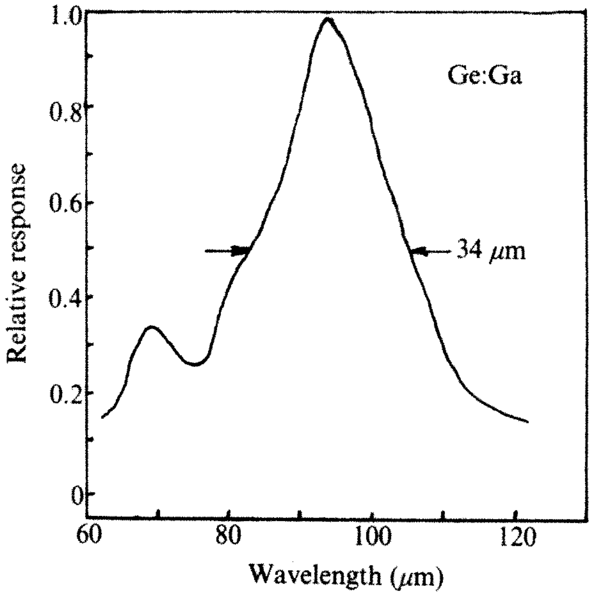
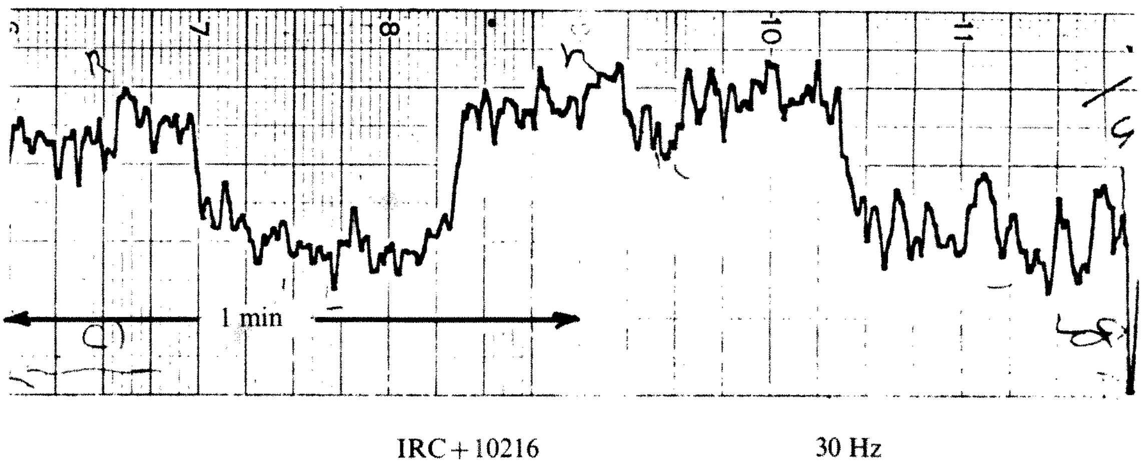
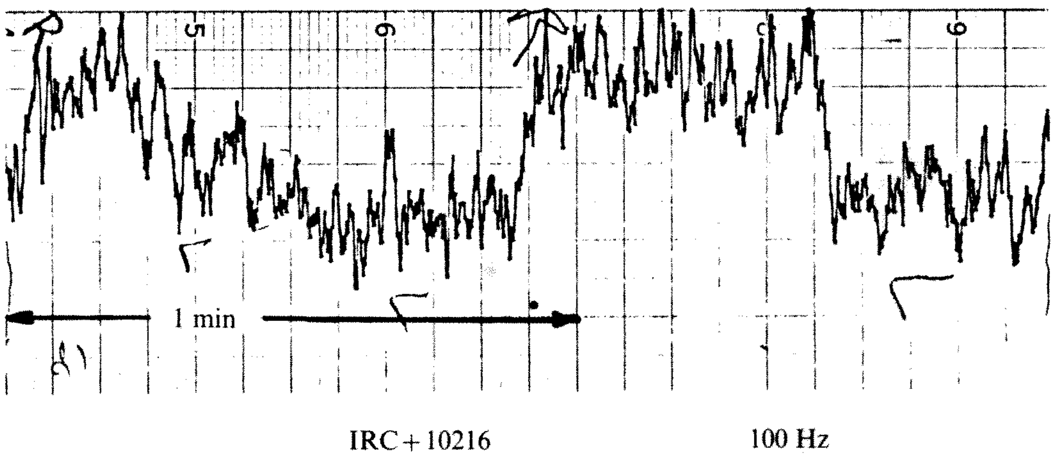


Fig. 1 *a*, Spectral response of Ge:Ga detector;



b, signal from IRC + 10216 at 30 Hz;



c, signal from IRC + 10216 at 100 Hz.

purposes for 5 min and IRC + 10216 for 30 min. Figure 1*b* and *c* shows the signals obtained from IRC + 10216 at modulation frequencies of 30 and 100 Hz; the two levels are produced by pointing the telescope so as to place the source in alternate beams. The apparent signal-to-noise ratio for both measurements was 6:1 but because of the wider recording bandwidth, the 100-Hz data are in fact less noisy when normalised to unit bandwidth. The flux level for IRC + 10216 was obtained by assuming a brightness temperature of 200 K for Mars, whose semi-diameter at the time of observation was 39.9″. Our 100-μm flux measurements of IRC + 10216 as shown in Table 1 agree with the measurements of Campbell *et al.*[4]. A unique aspect of this measurement is the use of

Table 1 100 μm flux measurement of IRC + 10216

Detector	Wavelength. λ_p (μm)	Bandwidth. $\Delta\lambda$ (μm)	Beam size (′)	Beam separation (′)	Measured flux (Jy)
Bolometer Univ. of Arizona	100	40	1.4	2	2,460
Photoconductor (Ge:Ga) N.R.L.	100	34	0.9	2	2,100

a photoconductive detector for the first time in an airborne infrared experiment. The sensitivity of our detection system is comparable with conventional bolometers that have been used in all airborne for infrared observations.

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2.4-µm mapping of the galactic central region

OBSERVATION of the diffuse galactic radiation at near infrared wavelengths where the interstellar extinction becomes comparatively small could be an effective way of investigating the galactic structure especially in the central region of the Galaxy. We report here observation of the diffuse galactic radiation at 2.4 µm in the central region of the Galaxy between $l = 350°$ and 30° using a balloon-borne telescope with a resolution of 1°. The central bulge of the Galaxy, and the very flat and narrow disk components were clearly demonstrated. A strong interstellar extinction concentrated in the galactic plane in the central region of the Galaxy was observed.

Our first observation by a balloon-borne telescope with wide spectral response (1.5–2.5 µm) was subject to unexpected temporal and spatial irregularities of the OH airglow emission[1]. For near infrared observation of the diffuse light at balloon altitudes we thus limited the wavelength range to between 2.35 and 2.45 µm; a unique band gap of the OH emission bands.

The successive observations were made on June 16, 1975 and May 31, 1976 by a 20-cm (f/1.25) balloon-borne telescope with two 5×5-mm² PbS detectors cooled by liquid nitrogen, each with a 1° square field of view, separated by 3.2° in elevation. The filters, which were also cooled, had an effective wavelength $\lambda_e = 2.4$ µm and a bandwidth $\Delta\lambda = 0.08$ µm.

A malfunction of the chopping motor in the flight of 1975 limited the intensity profile across the galactic plane at $l = 6°$. During the flight on May 31, 1976, we scanned the extended area along the galactic plane between $l = 350°$ and 30° within latitudes of $[b] \leq 10°$ in such a way that the elevation angle was stepped up by 0.9° after each strip scan of 30° width in azimuth.

The direction of the telescope was assigned to celestial coordinates with the aid of a geomagnetic aspectometer for the azimuth sensor and an elevation meter with a potentiometer. Definite detections of several bright IRC stars facilitated a check on the accuracy of the position determination, estimated to be ±0.3° throughout the observation.

The absolute responsivities of the detectors were calibrated by observing α Sco ($m_K = -3.87$) (ref. 2) and also monitored by an artificial calibration light during the flight. The contribution of nearby infrared objects, a single or a group of IRC stars, was subtracted by referring to *K*-magnitudes in the infrared catalogues[3,4].

The two detectors yielded independent values for the brightness distribution, and the consistency between these was satisfactory. The brightness values were synthesised into a map (Fig. 1). The data beyond $l = 27°$ may be less reliable because of a fault arising in the transmitter system, so the map has been limited within $l = 27°$. Our map tends to be smoothly linked to that presented by Hayakawa *et al.*[5].

The marked concentration of the surface brightness toward the galactic centre clearly implies that the central bulge or the spheroidal stellar distribution of our Galaxy has been detected. This constitutes the first demonstration of the deep inner structure of our Galaxy. The apparent shape of the central bulge must be subject to the interstellar extinction which should be highly concentrated in a narrow zone along the galactic plane. This is clearly indicated by the fact that the highest contours close to the galactic centre are prolonged not along the galactic equator, but perpendicular to it. The 2.4-µm brightness at the galactic centre is $1.6 \pm 0.03 \times 10^{-9}$ W cm^{-2} sr^{-1}.

Figure 2 shows the 2.4-µm brightness distribution along the galactic equator, the 2.6-mm CO line intensity[6] and the 100-µm brightness[7]. The near infrared profile seems to consist of two components, the central spheroidal structure and the flat disk component. Flatness beyond $l = 10°$ is remarkable, extending to the sudden decline near $l = 30°$, which was found by Hayakawa *et al.*[5]. This would imply a strong concentration of infrared sources in the 5-kpc ring as discovered from the CO observations[8]. Thinness of the disk component (4° in FWHM) is also prominent.

The concentration of the spheroidal component toward the galactic centre is much less steep compared with that observed in M31 in infrared[9,10], and in visual brightness distributions[11]. If the intrinsic brightness distribution of the central region of our Galaxy is the same as that of M31, the difference could be attributed to obscuration by a dense dust layer lying in the central region of the Galaxy. This dust layer extends mainly within $b = \pm 1°$, with a slight concentration towards the galactic centre. The total amount of the extinction in direction to the galactic centre is about 2.4 mag at 2.4 µm, corresponding to $A = 30$ mag, if we assume the general wavelength dependence of the interstellar extinction. It is almost compatible to the value obtained by Becklin and

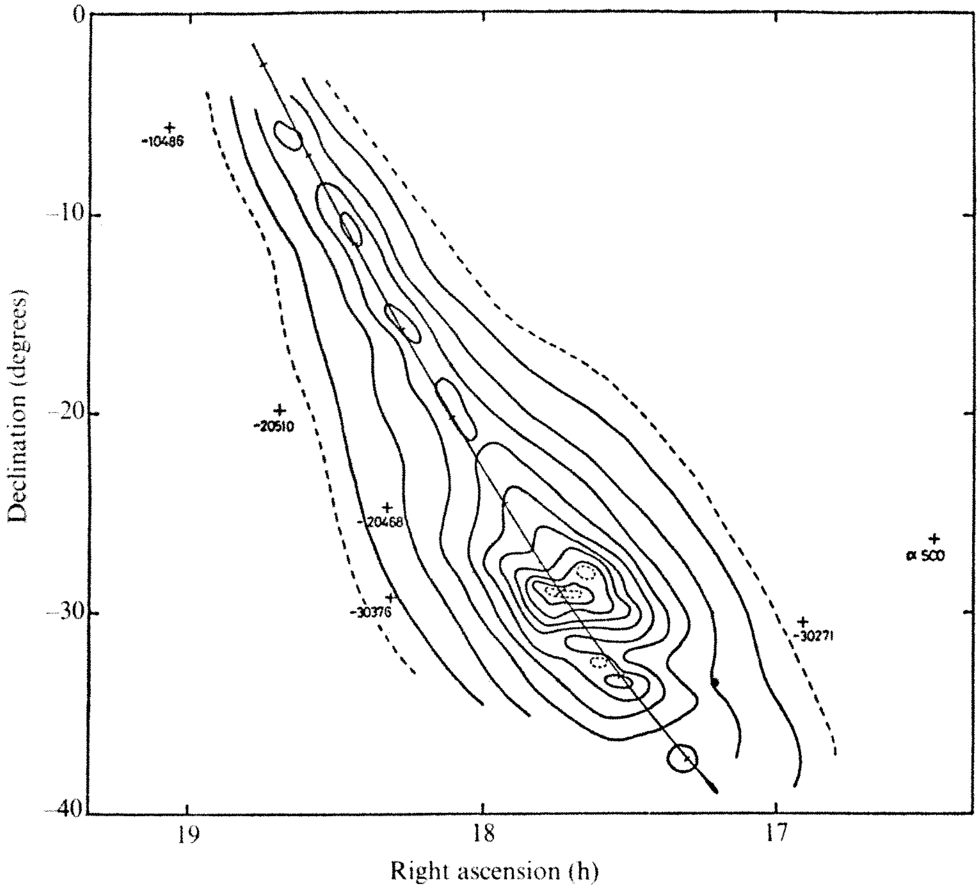
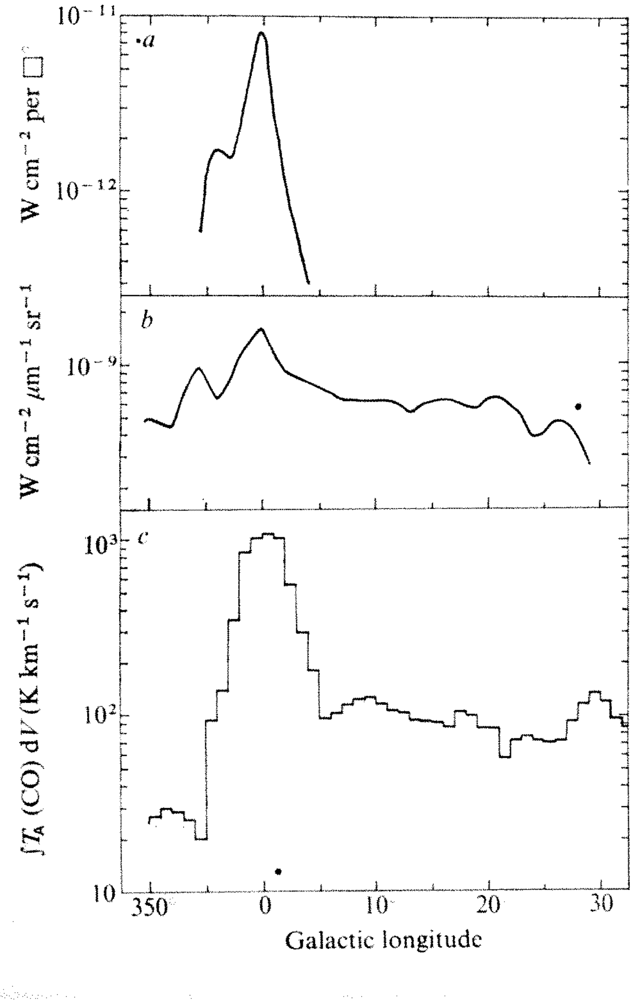


Fig. 1 2.4-μm map of the galactic diffuse light. Unit of the contour is 1.6×10^{-10} W cm^{-2} μm^{-1} sr^{-1}, dashed contours indicate half interval. Crosses indicate the positions of IRC stars used for the position calibration.

Fig. 2 *b*, Longitudinal dependence of the 2.4-μm intensity along the galactic equator. Those of *a*, the 100-μm intensity, and *c*, the CO intensity ($J = 1 \rightarrow 0$) are shown for comparison.



Neugebauer[12]. About one third of the extinction is attributed to the dust layer in central region of the Galaxy, that is, within $l = \pm 2°$. This dust layer corresponds well to that detected as the extended far infrared source by Hoffmann *et al.*[13] and Soifer and Houck[7], and may be related to the CO molecular clouds concentrated in the galactic centre.

It is noticeable that there is an asymmetry between positive and negative sides of the galactic longitude. A dip near $l = 357°$ may correspond to the subsidiary peak in the 100-μm profile. A peak near $l = 354°$ is also interesting. Open clusters NGC 6383 and 6405 (M 6) locate at the same position but seem to be insensitive to the 2.4-μm brightness. Similar features have been found in the balloon observation made by the infrared group of Nagoya University (T. Matsumoto, personal communication).

Whether these features are caused by variation in local interstellar obscuration or by some hidden objects should be clarified by further investigation.

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Infrared profile of central region of our Galaxy at 2.47 μm

SEVERAL investigators have described the features around the galactic centre in some infrared wavelength regions[1-3]. Their results demonstrate the conspicuous peak and several humps around it. These observations, however, are confined to a rather small region mainly because of the terrestrial background radiation. So the overall feature of the central bulge of our Galaxy could not be derived. Studies of the central bulge have been carried out only with the aid of the distribution of RR Lyrae variables through the 'galactic window'[4], but the distribution does not necessarily represent that of general stars which are responsible for the galactic structure. On the contrary, near infrared observation reveals the structure of the central bulge more directly, because the main constituents of the bulge are probably late-type stars which radiate their energy effectively in the infrared region, as inferred from external galaxies. Much weaker interstellar extinction in the infrared region also enables us to observe the whole central bulge through thick interstellar dust clouds. The surface brightness at 2.4 μm in the range of $75° > l > 23°$, $|b| < 20°$ was attained by our previous balloon experiment[5,6]. We have now attempted further similar observations aiming mainly to derive the surface brightness of the central bulge of our Galaxy.

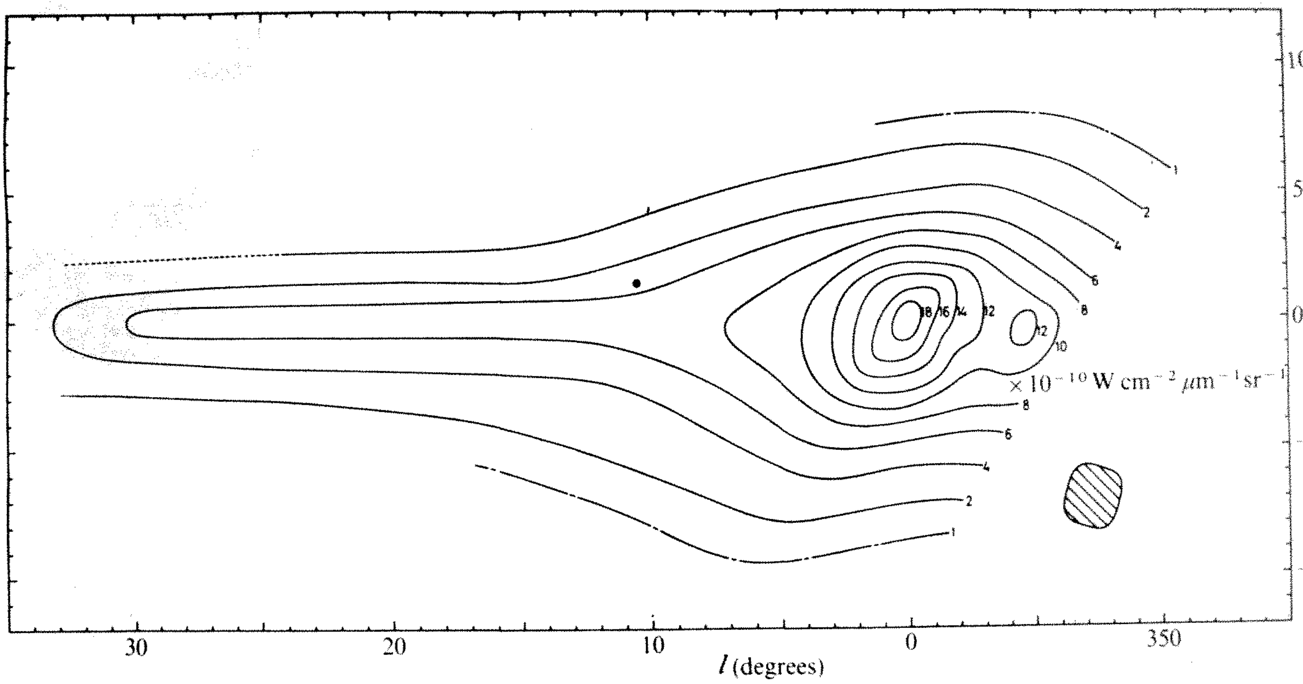


Fig. 1 Isophoto of the observed surface brightness of our Galaxy. The shaded area (bottom right) indicates the field of view used (HMBW).

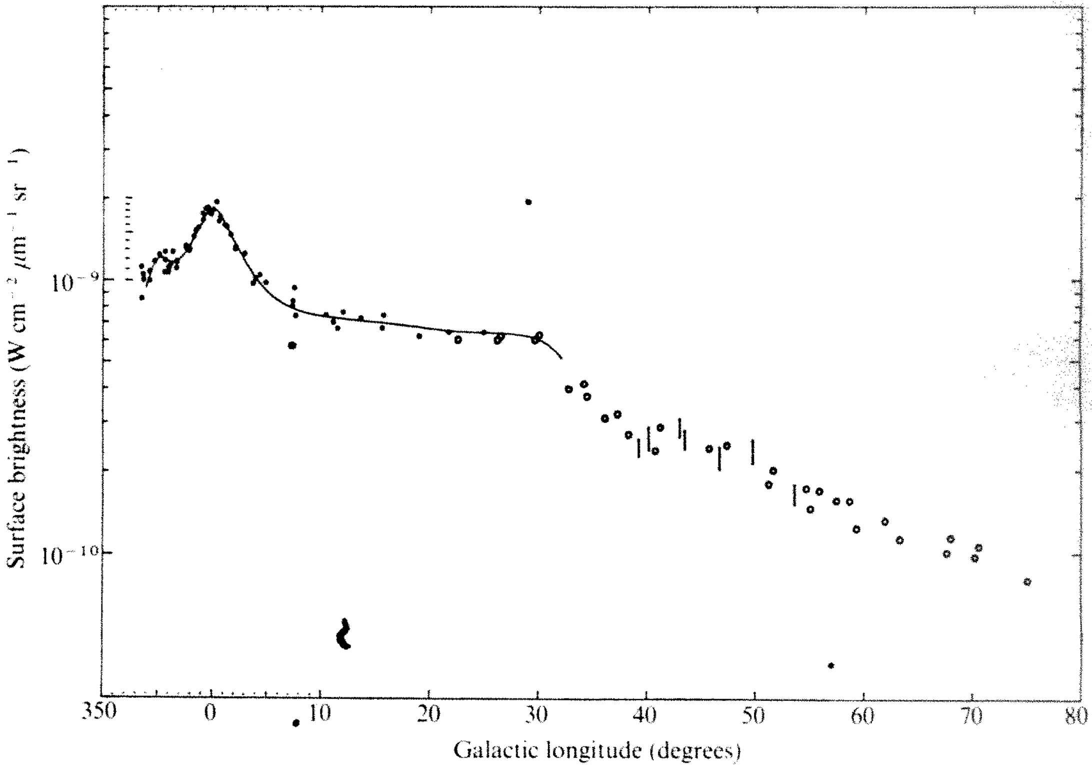


Fig. 2 Longitude dependence of 2.4-μm surface brightness on the galactic plane. Newly obtained data are shown by filled circles while open circles and bars represent the previously observed data[5,6]. The solid line is drawn according to the isophoto.

The balloon-borne infrared telescope was similar to that used for the previous flight. It consisted of a silicon combination lens system (D = 10 cm, F = 1.0) in front of which an interference filter (λ = 2.4 μm, $\Delta\lambda$ = 0.1 μm) was attached, and an array of four lead sulphide detectors installed at the focal plane, each of which generated the field of view of 2° × 2°. The whole system was cooled by liquid nitrogen to reduce the thermal background radiation and improve the detector sensitivity.

The instrument on board a balloon was launched at 1850 JST on May 30, 1976 from Sanriku Balloon Center of the Institute of Space and Aeronautical Science, University of Tokyo.

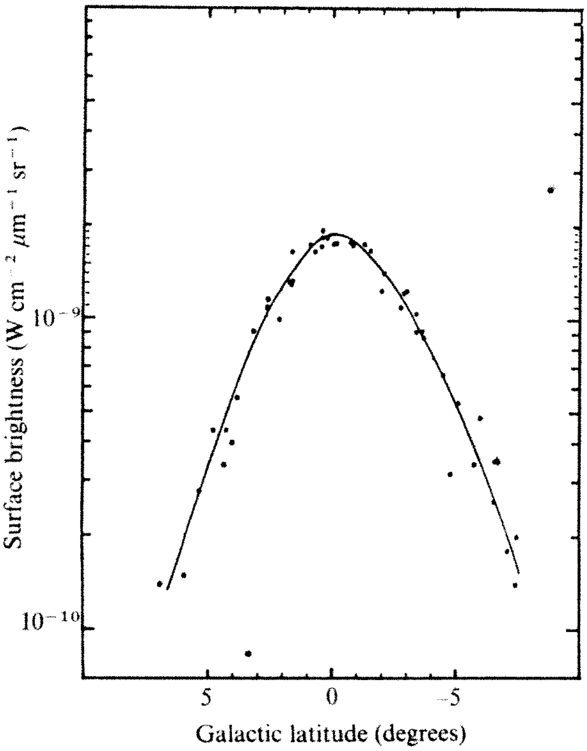
The whole system functioned as expected during the flight and a celestial region of $35° \geq l \geq -7°$, $|b| < 15°$ was covered by 72 scans. These scan paths were derived from the output of the magnetometer and confirmed by the transit of α Sco across the field of view. The observation of α Sco also provided an absolute calibration. The flux 1.38×10^{-12} W cm^{-2} μm^{-1} of α Sco at 2.4 μm was provided by the balloon observation on the previous night[7]. The responsiveness of the detector systems was checked by lighting a calibration source.

The infrared surface brightness thus obtained is shown in the form of an isophoto in Fig. 1. The scan paths are rather concentrated into the range of $-10° < l < 10°$, so that the contour lines in that region are more accurate than in the rest. The contours beyond l = 30° are drawn with reference to previous data. The longitude dependence at b = 0° and latitude dependence at l = 0° are shown in Figs 2 and 3, respectively. The present result agrees well with our previous one between l = 20° and 25°.

At a glance the isophoto shows that the galactic radiation at 2.4μm consists of two components: disk component and bulge component. The surface brightness and width of the plane are nearly constant in the region $10° < l < 30°$ with the value of 6.5×10^{-10} W cm^{-2} μm^{-1} sr^{-1} and 4.5° (f.w.h.m.) respectively.

The bulge component extends in the region $l < 5°$ which corresponds to 1 kpc from the centre. If we exclude the hump at l = 355.5°, b = −0.5° and absorbing lane from centre to l = 357.5°, b = −2.5°, the bulge component is almost symmetrical with respect to the galactic centre.

Fig. 3 Latitude dependence of 2.4-μm surface brightness at l = 0°.



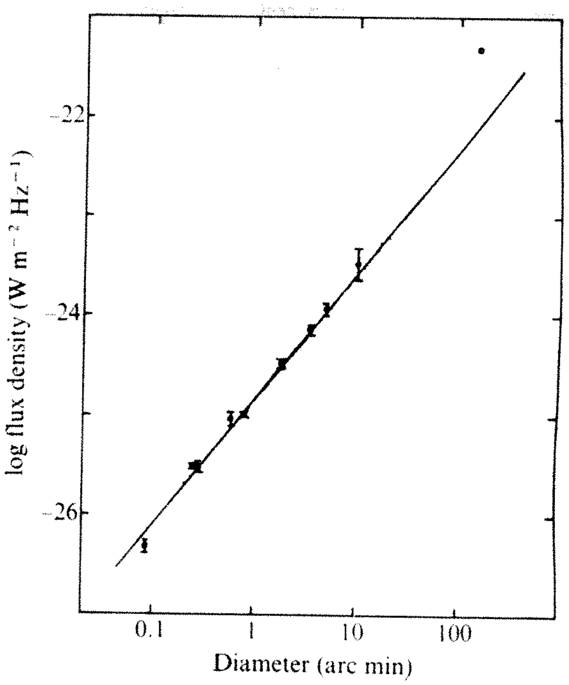


Fig. 4 The relation between flux density of the galactic centre and aperture diameter employed. Filled circles and straight line are taken from ref. 1. An open circle at upper right represents our value.

The flux of the galactic centre of $(1.9 \pm 0.1) \times 10^{-9}$ W cm^{-2} μm^{-1} sr^{-1} is compared with ground-based observations at 2.2μm[1] in Fig. 4. Our value is rather larger than that extrapolated from the small aperture, since our value contains the disk component and the large aperture results in smaller interstellar extinction because of strong concentration of the absorbing matter on the galactic plane.

The contour lines within 2 arc deg from the centre in Fig. 1 are elongated in the direction perpendicular to the galactic equator, and in the outer region the lines are elongated parallel to the galactic plane. The former can be attributed to the interstellar absorption, and the latter probably reflects the property of the central bulge.

The hump at l = 355.5°, b = −0.5° in Fig. 1 is considered to be an extended source, since there are no corresponding bright stars at 2.2 μm around the position of the hump[8]. The open cluster NGC 6383, which is located at the almost same position, cannot contribute to the 2.4-μm surface brightness. A radio source which may correspond to the hump is not also found in this region. It is uncertain at the present time, however, whether the hump is related to the structure of the central bulge.

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Disappearance of puddle water during the night with growth of ice needles and its reappearance during the day

A PUDDLE appears in a dip in the ground after a fall of rain or snow. It becomes covered with an ice sheet during cold nights in the winter in the rural area of the northern part of the Kanto district in Japan. The ice sheet has a characteristic shape. It is not flat but dished and usually an air space is found under thin ice sheets without puddle water. Furthermore, an abundance of ice needles grow on the soil surrounding the puddle. These phenomena in puddles in winter seem to be general in the Kanto district. This report describes observations made on some puddles located at the side of the road in the Kanto district during February 1976 to clarify the process of formation of the dished-out ice sheet, the air space under the ice sheet, and the disappearance and appearance of puddle water.

Photographs of the various states of puddle water obtained on February 7–8 are shown in Fig. 1*a-f*. In the daytime the puddles were not covered with an ice sheet (Fig. 1*a*) though they were covered with ice sheets early in the morning before the increase in air temperature (Fig. 1*b*).

Stripe patterns were found in the ice sheet. Under the ice sheet the puddle water was replaced by air (Fig. 1*c*). A cross section of the ice sheet showed the difference of ice thickness with position (Fig. 1*d*). The stripes observed on the ice sheet are due to the difference in ice thickness.

Abundant ice needles 3–5 cm long were observed on the soil surrounding the puddle (Fig. 1*e*). Furthermore, melting of the ice needles and an increase in puddle water under the ice sheet were observed in the morning with the increase in solar radiation.

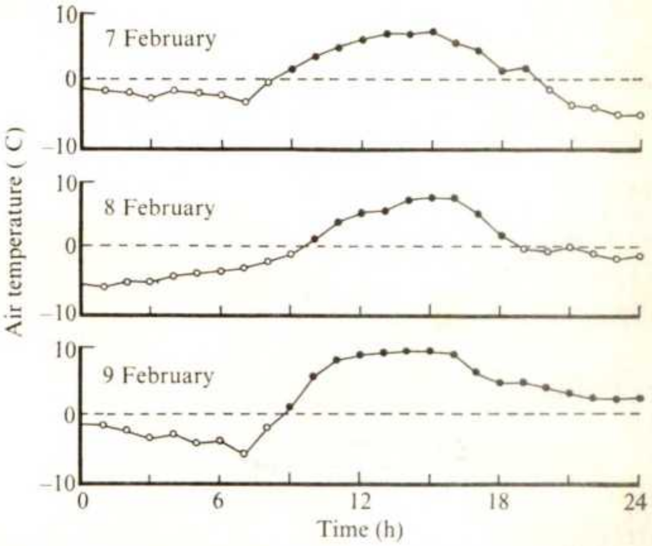
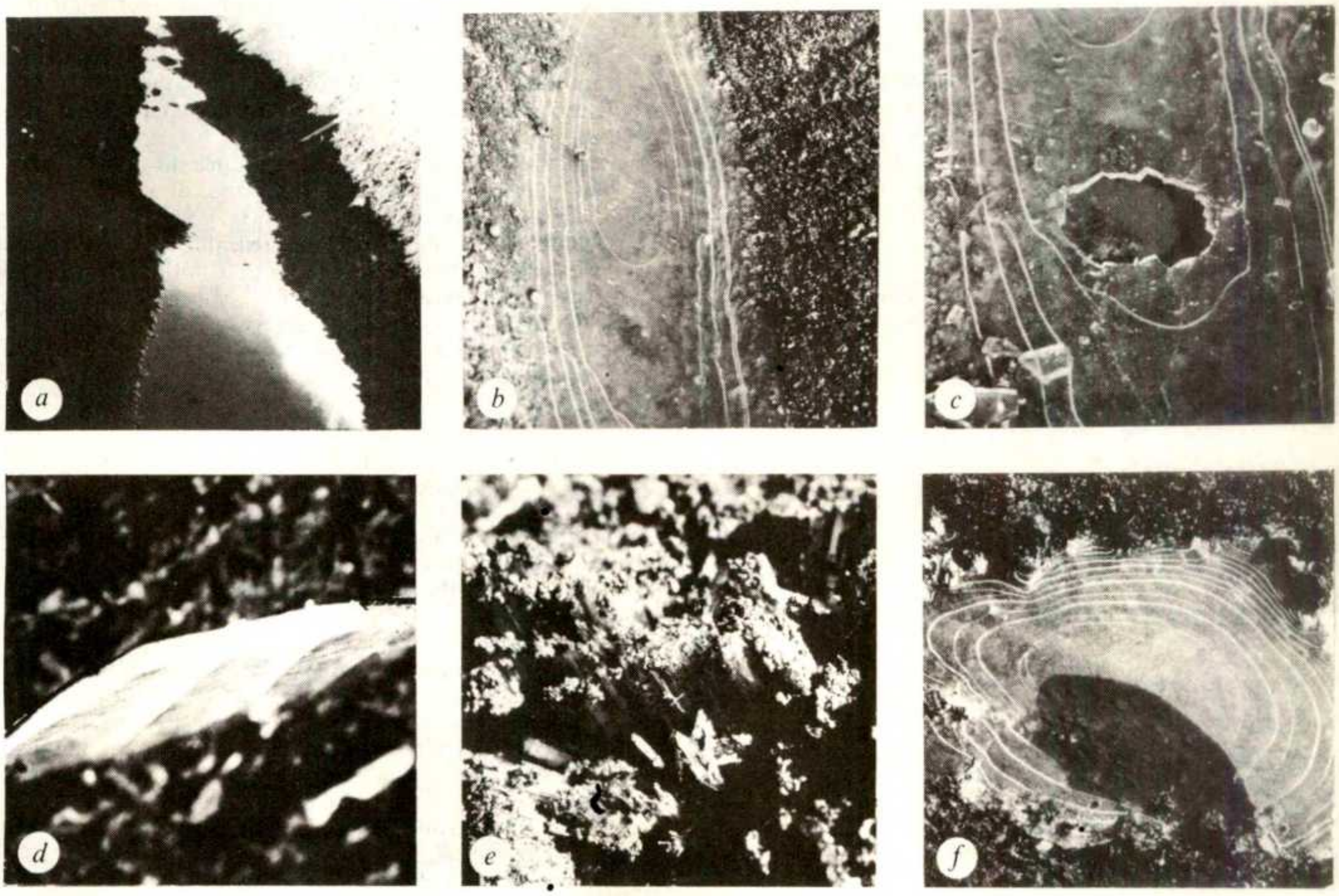


Fig. 2 Air temperature variation about 300 m from observation area.

The air temperature data obtained at the Tateno Aerological Observatory about 300 m from the observed puddles on February 7–9, 1976 showed the cycle of air temperature above 0 °C from 0900 to 1900 and below 0 °C from 2000 to 0800 (Fig. 2).

These observations suggest that the following events occur. The growth of ice needles around the puddle is due to the supply of water from the puddle followed by the

Fig. 1 Photographs of puddle water at various times of day.



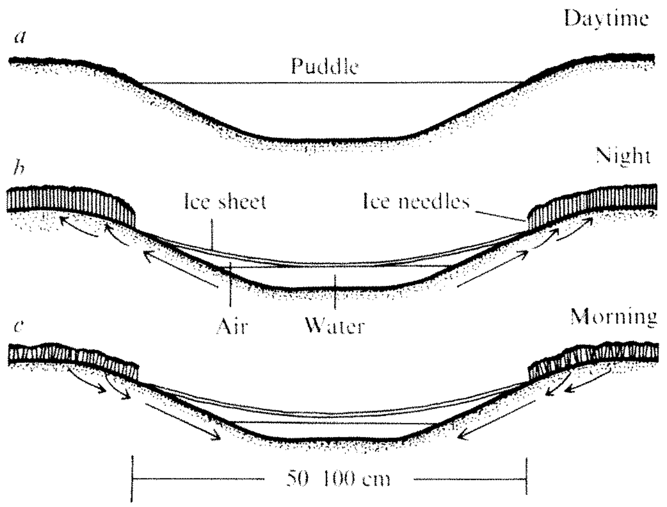


Fig. 3 Air space formation beneath puddle ice sheet.

decrease of puddle water. The decrease of puddle water results in the gradual lowering of the level of formation of the ice sheet which occurs on the surface of puddle water. This is the reason for the formation of the dished ice sheet. And then, with the continuation of the growth of ice needles, the puddle water separates from the ice sheet to form an air space (Fig. 3). The irregularity of the growth of ice needles controlled by the delicate balance of meteorological conditions of air and soil[1-7] may be the cause of the striped patterns of the ice sheet. With the increase of solar radiation in the morning, the ice needles and ice sheet melt and water returns to the puddle.

After the melting of the ice needles and ice sheet, the puddle water regains its previous daytime shape.

The puddles formed at the side of road after a fall of rain or snow in winter repeat this process with the evaporation of puddle water. The area of water evaporation from the surface of puddle water is extended by the penetration of the water into the soil surrounding the puddle. This results in more rapid disappearance of puddle water from the dip in the ground. The periods of existence of the puddles which we observed were about 10 days.

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Measurement of forces of molecular attraction of crossed fibres as a function of width of air gap

DERJAGUIN and Abrikosova[1] were the first to measure molecular forces as a function of the distance between macro-objects, using negative feedback. The results coincided, within the limits of experimental error, with the conclusions of the theory of retarded molecular forces (which was subsequently developed by Lifshits[2,3]). In the earlier research work[1], as in a number of subsequent experiments, the width of an air gap was measured by optical methods (using the interference of light). The research work was therefore limited to transparent bodies. Moreover, test bodies had to possess a sufficiently large radius of curvature. The latter condition meant that the quality of the surfaces had to meet stringent requirements; it was also difficult to ensure the absence of dust particles in the gap between interacting bodies with large radius of curvature. As a result, only in two cases[4,5], owing to the use of molecular-smooth surfaces of mica, did researchers succeed in measuring, not only the retarded, but also non-retarded molecular forces.

In the present work, we have used fibres of small radius (about 150–500 μm) crossed at right angles. With a view to presetting the gap, instead of measuring it directly (including that between opaque bodies), we preset the displacement of one of those bodies relative to the position of the contact with another fixed body. (The contact we assumed to be that position of fibres, which corresponds to the contact interaction force $F = 0$). To this end, a movable fibre was attached to the frame of a photoelectric galvanometer and another fibre was fixed.

The light ray reflected by the galvanometer mirror impinged on two photoresistances. When the instrument was operating normally as a galvanometer, the equilibrium position of the frame corresponded to the equality of illumination of those photoresistances. In the case of a deviation from the equilibrium, current I_1 passed through the diagonal of the bridge circuit, and a part of current I_1, current I_2 through the galvanometer frame. Owing to the negative feedback, the current I_2 reset the frame in its equilibrium state. In addition to the two photoresistances, the bridge circuit comprised two constant resistances S_1 and S_2. When the frame was in equilibrium, $I_1 = 0$.

The instrument was remodelled by replacing the constant resistances S_1 and S_2 by the variable ones, to measure the forces of interaction of fibres. To this end, multistage magazines of resistances were used. By varying S_1 and S_2, one could vary the illumination ratio of the photo-resistances, corresponding to $I_1 = 0$, and, hence, the equilibrium position of the frame. The readings S_1 and S_2 were converted into the values of displacements of the movable thread by the preliminary graduation. The currents I_1 and I_2 were measured by microammeters.

Each equilibrium position of the movable thread was preset by the values of S_1 and S_2; in the absence of a force applied to the thread this corresponded to $I_1 = 0$.

When the interaction forces appeared, this equilibrium state was violated. An electric current $I_1 \neq 0$ flowed through the diagonal of the bridge circuit. To compensate for it, an independent controllable power source was used, which was additionally cut in the circuit of the galvanometer frame. The value of the current, I_2, was proportional to the forces applied to the thread. The instrument had been previously graduated, to convert the values I_2 into the force value.

Thus, the space position of the moveable fibre, which was read off the contact point of fibres, was determined by the values S_1 and S_2 at $I_1 = 0$. In this case, the force of interaction of the fibres was determined according to the current I_2. Consequently, the circuit applied enabled us, using only one device, to measure both the spacings between the fibres and their interaction forces.

As a result, we have succeeded in presetting the shift of fibres at an accuracy of up to 10 Å. The position of the contact was defined at an accuracy of up to 50 Å. The sensitivity of the instrument to the interaction force was about 10^{-4} to 10^{-5} dyne.

The forces of interaction of quartz, glass, platinum and gold fibres were measured as a function of the shortest distance h between them, within the range of 100–1,000 Å. In the case of dielectrics, a β-radiation source was used to remove electrostatic charges.

The theory of molecular forces[2,3] gives the following relationships for the forces of attraction of cylinders crossed at right angles and having radii R_1 and R_2

$$F_1 = 2\pi B\sqrt{(R_1R_2)}/3h^3 \quad (1)$$

$$F_2 = A\sqrt{(R_1R_2)}/6h^2. \quad (2)$$

Equation (1) corresponds to the case of retarded forces, and equation (2) to that of non-retarded forces. Here A and B are the constants of molecular forces.

To compare the experimental results obtained with fibres of different diameters, it will be more convenient to use the interaction energy u per unit area of the plane-parallel surfaces separated by the same gap h. In accordance with ref. 6,

$$u = \frac{F}{2\pi\sqrt{(R_1R_2)}};\quad u_1 = \frac{B}{3h^3};\quad u_2 = \frac{A}{12\pi h^2} \quad (3)$$

Unlike F, the values u do not depend on the radius of the cylinders that are used to measure the force.

Figure 1 shows the relationships $u(h)$ obtained for quartz and platinum fibres. Each point on the curve represents a result of averaging several experimental values of u with a definite value h. Solid straight lines were plotted by the least squares method (over the entire field of points, but not according to the averaged results), taking into account the square or the cube dependence of u on $1/h$.

Using the same plurality of experimental points as the basis, we were able to determine the exponent n, without having recourse to the relationships (1) and (2) (assuming that $u \sim 1/h^n$). For quartz, the values n were found to be equal to $n_1 = 3.01$ and $n_2 = 2.0$; for platinum, to $n_1 = 3.03-3.23$ and $n_2 = 1.93-2.01$. This agrees well with theory. At $h \lesssim 300$ Å, platinum deviates from the dependence $u \sim h^{-2}$; this is probably attributable to the roughness of the surface of fibres.

From the results represented in Fig. 1, it was easy to determine the values of constants A and B, as well as to evaluate the gap width h_0, at which the non-retarded molecular forces go over into the retarded ones.

Table 1 gives the values of A, B and h_0 for quartz and platinum. The values of A and B obtained agree satisfactorily with the calculated ones[7]. In the case of quartz, those values are close to the measured results obtained by other methods: $A = 6.5 \times 10^{-13}$ erg, $B = (0.66-1.15) \times 10^{-19}$ erg cm (ref. 6). We were the first to obtain the experimental values B for platinum. The values A for platinum were calculated earlier, using the results of experiments made with crossed fibres in electrolyte solutions[8]: $A = (0.8-1.6) \times 10^{-12}$ erg.

Fig. 1. Dependence of the specific energy of the molecular attraction of plane-parallel surfaces, u, on distance h. a, Quartz, $2R_1 = 0.8$ mm, $2R_2 = 1.15$ mm. b, Platinum, $2R_1 = 1$ mm, $2R_2 = 0.3$ mm. c, Platinum, $2R_1 = 2R_2 = 0.3$ mm.

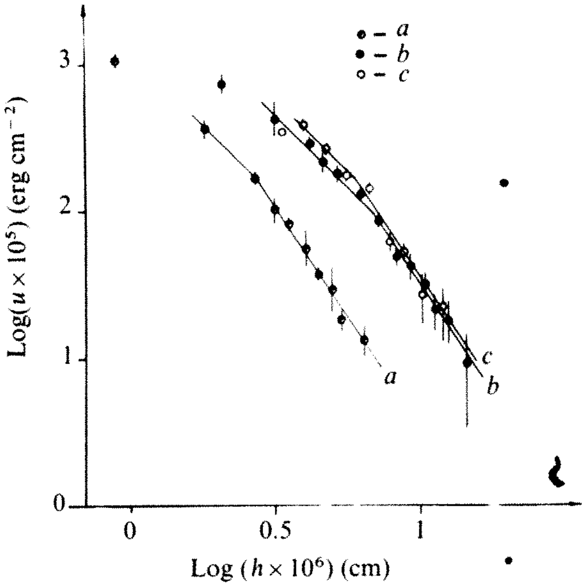

Table 1 Results of measurements of A and B

Material	$A \times 10^{12}$ (erg) Expt	$A \times 10^{12}$ (erg) Theory	$B \times 10^{19}$ (erg cm) Expt	$B \times 10^{19}$ (erg cm) Theory	h_0 (Å)
Fused quartz	0.45	0.8	1.02	0.6	270
Platinum	2.0	—	10.5	13.0	650

Hence, the method thus worked out allowed the molecular forces to be measured as a function of the spacings between the fibres in air at a distance of about 100–1,000 Å between various materials, including these opaque ones. The experimental results agree well with the theory.

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Cross relaxation and spin diffusion in the proton NMR of hydrated collagen

PROTON NMR of water is being used extensively to probe the molecular dynamics of water molecules in biological systems such as protein solutions, hydrated macromolecules, cells and tissue. The nuclear magnetic relaxation rates R_1 (spin–lattice) and R_2 (spin–spin) can be analysed in terms of the rotational motions of the water molecules[1–3]. A crucial assumption in this analysis is that the proton relaxation of the water proceeds independently of that of the macromolecules. Kimmich and Noack[4–6] claim that this assumption may be incorrect for proton spin–lattice relaxation because of spin diffusion, but clear evidence of spin diffusion in hydrated biological samples has not been reported. We show here that the proton spin–lattice relaxation behaviour in hydrated collagen is dominated by cross relaxation between the water protons and the macromolecular protons as a result of spin diffusion; the macromolecular spin–lattice relaxation contributes significantly to the water proton R_1.

The proton spin–lattice relaxation of ribbons of reconstituted collagen[7], hydrated with H_2O or D_2O, was studied by pulsed NMR. The spin–lattice relaxation was determined from a comparison of the free induction decay (FID) signal after a (180° – t – 90°) pulse pair with that after a single 90° pulse. The pulse pair yields the partially relaxed magnetisation $M_z(t)$ whereas the single 90° pulse yields the equilibrium magnetisation M_0. The spin–lattice relaxation rate was determined from a logarithmic plot of the reduced magnetisation $m(t) = -(M_z(t)-M_0)/2M_0$ against t.

The FID of hydrated collagen consists of two components (see Fig. 1). Protons in the rigid collagen matrix constitute a

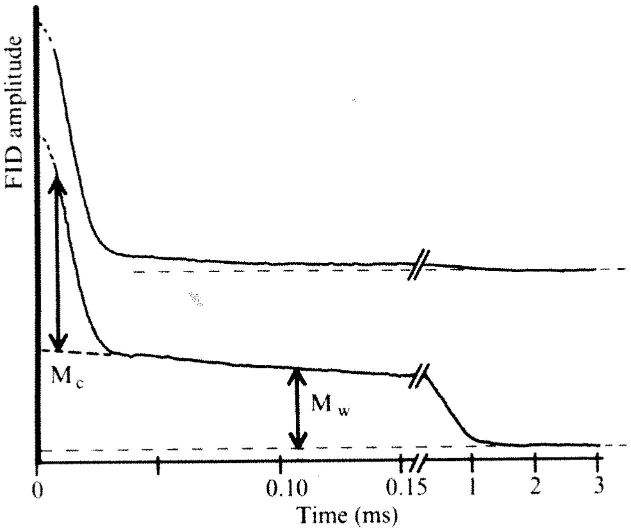

Fig. 1 Proton FID signals in reconstituted collagen, hydrated with 28 g water per 100 g collagen. Lower curve: hydrated with H_2O. Upper curve: hydrated with 95% D_2O. The collagen signal intensity $M_c(t)$ was measured immediately after receiver recovery from the 90° pulse, 10 μs after the beginning of the pulse. The water signal intensity $M_w(t)$ was measured 60, 110 and/or 400 μs after the pulse; the spin–lattice relaxation decay at these times is the same. The collagen fibres were oriented perpendicular to the magnetic field. Measurements were performed at room temperature with a Bruker pulse spectrometer operating at 30 MHz; a Varian V-2708 magnet was used, equipped with a Varian V-3508 flux stabiliser. The 90° pulse length was 1.5 μs. The FID signals were averaged with a combination of a Biomation 802 signal digitiser and a Nicolet 7002 signal averager. Typically 64 or 128 repeated measurements were averaged together.

fast relaxing part ($R_2^* = 60{,}000\ s^{-1}$), and the water protons relax much more slowly ($R_2^* \lesssim 5{,}000\ s^{-1}$). This identification of the two signal components is supported by the observations that the intensity of the water fraction changes in direct proportion to the amount of hydration water and to the isotopic dilution with D_2O, and that the relative intensities of the two fractions correspond to the calculated ratio of collagen protons to water protons (three to seven, respectively, for the sample shown in Fig. 1). No static dipolar splitting of the water signal was apparent, in agreement with other studies of natural collagen under similar conditions[8,9].

The two components of the FID can easily be separated (see Fig. 1) so that we could study the spin–lattice relaxation of the water and of the macromolecular protons separately (Fig. 2). Measurements at different water contents all showed the following characteristics (see Fig. 2): (1) The relaxation rate R_1 of both water and collagen protons is the same if the initial 10 ms of the relaxation decays is ignored. (2) The beginning of the water relaxation decay is clearly concave (curve *c*) and that of collagen is convex (curve *b*). Both relaxation curves are described by a sum of two exponentials. (3) When collagen is hydrated with D_2O, then the relaxation rate of the collagen protons is single-exponential and proceeds at a rate which is much slower than in the presence of H_2O (curve *a*).

Proton NMR relaxation is brought about by magnetic dipolar interactions. Substitution of D_2O for H_2O changes the nuclear dipolar interactions between collagen protons and water nuclear spins. Assuming that the dynamics of hydration are similar for H_2O and D_2O, then the dipolar interaction between a collagen proton and a water proton is of the order of $(\gamma_H/\gamma_D)^2\ (I_H/I_D) \times (I_H+1)/(I_D+1) = 16$ times more effective at inducing nuclear spin transitions than the interaction between a collagen proton and a water deuteron[10]. The large change in R_1 of the collagen protons on substitution of D_2O for H_2O clearly indicates that the macromolecular protons 'feel' the presence of water protons. Consequently, dipolar coupling between water and macromolecular protons must be an important factor in the proton relaxation mechanism in hydrated collagen.

The non-exponentiality of the water relaxation might be ascribed to the presence of two slowly exchanging water fractions. This seems unwarranted, however, as it does not explain the convex curvature in the collagen relaxation, or account for the similar relaxation rates of the two components after the initial 10 ms of decay. We can explain all of our observations by realising that the dipolar coupling between water and collagen protons provides a way for cross relaxation between the water and the collagen protons by the spin diffusion mechanism[10,11].

We will assume that the protons in the water phase as well as in the collagen phase have, at any time, a uniform spin temperature, that is, the longitudinal magnetisation $M_z(t)$ in each phase is uniform. Within the collagen phase spin diffusion among the collagen protons will rapidly establish a common spin temperature, whereas in the water phase this is accomplished by rapid chemical exchange. The water phase may also include exchangeable collagen protons. Let p_w and p_c be the fraction of protons in the water phase and in the collagen phase respectively, with the proton relaxation rates in the absence of cross relaxation denoted by R_{1w} and R_{1c}. It should be noted that R_{1w} comprises the contributions to the water proton relaxation rate which are normally included in theoretical treatments, for example, effects from bound and free fractions of water[1–3]. Whenever a proton of the water phase resides near a collagen proton, the two protons are coupled by the magnetic dipolar interaction and a mutual spin flip can occur[10]. Such a spin flip exchanges spin energy between the two proton phases,

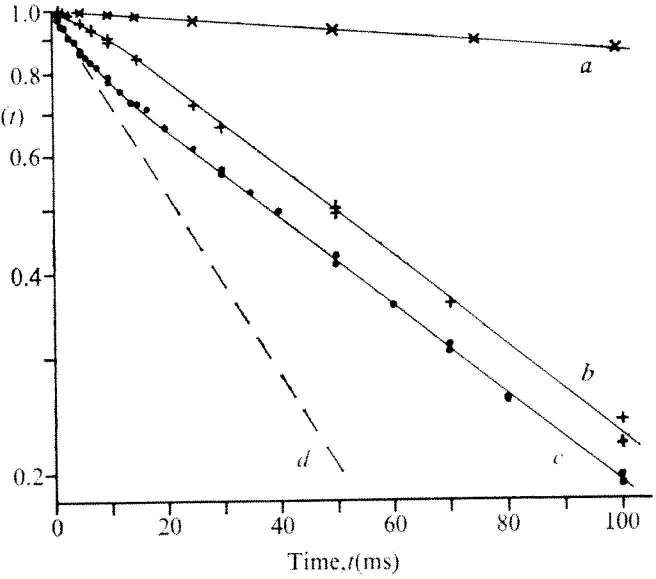

Fig. 2 Proton spin–lattice relaxation decays for the reduced magnetisation $m(t)$ in hydrated reconstituted collagen for the same samples as in Fig. 1. Measurements of $M_z(t)$ and M_o were alternated and the FID signals were averaged into two halves of the memory of the signal averaging system. This alternation ensures very accurate R_1 measurements since slow changes in time of signal gain and/or of field drift affect $M_z(t)$ and M_o in the same way and are compensated. Curve *a*, $m_c(t)$ for the collagen proton signal, hydrated with 95% D_2O (×). Curve *b*, $m_c(t)$ for the collagen proton signal, hydrated with H_2O (+). Curve *c*, $m_w(t)$ for the water proton signal of H_2O (●). A least squares fit of equation (2) to $m_w(t)$ yields the estimates, $R_1^- = 15 \pm 1\ s^{-1}$; $c_w^- = 0.89 \pm 0.01$; $R_1^+ = 160 \pm 15\ s^{-1}$; $c_w^+ = 0.11 \pm 0.01$. Curve *a* gives $R_{1c} = 3 \pm 0.5\ s^{-1}$. With $p_w = 0.3$, equation (3) gives $R_{1w} = 31 \pm 4\ s^{-1}$; $k_c = 40 \pm 10\ s^{-1}$; $k_w = 100 \pm 25\ s^{-1}$; $c_c^- = 1.08 \pm 0.01$; $c_c^+ = -0.08 \pm 0.01$. Solid lines represent the theoretical decays obtained with these values of the parameters. Curve *d* represents the water relaxation decay with a relaxation rate $R_{1w} = 31\ s^{-1}$ as it would occur in the absence of cross relaxation.

leading to cross relaxation between the two phases. Spin diffusion may occur through protons on water molecules that are temporarily bound to the collagen, or through rapidly exchanging collagen protons. Spin diffusion will be effective in particular, if such bound nuclei exchange at a rate which is slow compared to ω_0, the NMR measuring frequency[10,11].

We can introduce a cross relaxation rate k_c, the rate at which magnetisation diffuses from the collagen to the water; k_w is the reverse rate. We can then write a set of modified Bloch equations, similar to those well-known for the case of chemical exchange:

$$\mathrm{d}m_w(t)/\mathrm{d}t = -R_{1w}m_w(t) - k_w m_w(t) + k_w m_c(t) \qquad (1a)$$

$$\mathrm{d}m_c(t)/\mathrm{d}t = -R_{1c}m_c(t) - k_c m_c(t) + k_c m_w(t) \qquad (1b)$$

Unlike the case of chemical exchange, (1) applies only to the z-component of the magnetisation; spin diffusion does not lead to cross relaxation in the transverse magnetisation.

The solution of the set of coupled differential equations (1) predicts that the relaxation in either phase is described by two apparent relaxation rates R_1^+ and R_1^- which are the same for both phases. The complete solution[12] is (the subscript i refers to either of the phases)

$$m_i(t) = c_i^+ \exp(-R_1^+ t) + c_i^- \exp(-R_1^- t) \qquad (2)$$

where

$$R_1^{\pm} = \tfrac{1}{2}(R_{1c} + R_{1w} + k_c + k_w) \pm \tfrac{1}{2}\sqrt{[(R_{1c} - R_{1w} + k_c - k_w)^2 + 4k_c k_w]} \qquad (3a)$$

$$c_i^{\pm} = \pm(R_1^{\mp} - R_{1i})/(R_1^- - R_1^+) \qquad (3b)$$

$$k_w = (p_c/p_w)k_c \qquad (3c)$$

From (3b) follows

$$R_{1i} = c_i^+ R_1^+ + c_i^- R_1^- \qquad (3d)$$

With these expressions all the characteristics of the spin–lattice relaxation can be explained quantitatively.

The two fractions in the relaxation curve of the water signal are clearly resolved, permitting the determination of all the parameters in equation (2). A fit to the data points (see Fig. 2) yields R_1^+, R_1^-, c_w^+ and c_w^-; using these values, R_{1w} is calculated, from equation (3d), to be 31 ± 4 s^{-1}. A similar calculation for the collagen proton signal decay is less accurate, but gives $R_{1c} \approx 3\ \mathrm{s}^{-1}$, which is similar to R_{1c} measured in D_2O. p_c and p_w can be determined from the relative signal intensities in the FID, extrapolated to zero time (Fig. 1), or from the known amounts of protons in the two phases. Within experimental error the two methods give the same values for p_c and p_w.

The parameters that describe the complete relaxation behaviour consistent with equations (3) are given in the legend to Fig. 2. For the cross relaxation rate k_c we obtain an estimate of 40 ± 10 s^{-1} for a hydration of 28 g H_2O per 100 g collagen. We believe that this is the first time that a figure has been given for the proton spin diffusion rate from a protein to the hydration water. Our measurements show that the water phase constitutes the relaxation 'sink' for the collagen proton phase. This result differs with the model of Kimmich and Noack[4–6], who proposed that the water relaxation proceeds via relaxation 'sinks' located with the macromolecular phase.

Usually, water relaxation rates in hydrated biological systems are obtained from plots similar to Fig. 2. It is common practice, however, to ignore, or avoid measurements (because of associated experimental difficulties) during the initial 10 ms of the relaxation decay. It is clear from our analysis that this practice would lead to a measurement of an apparent relaxation rate of water (R_1^- in the present case), which is not necessarily the same as R_{1w}. We find for the hydrated collagen sample in Fig. 2 that $R_{1w} \approx 2R_1^-$. Clearly, cross relaxation can significantly affect water proton spin–lattice relaxation in biological samples. Its importance must be more carefully evaluated before applying molecular dynamical interpretations to apparent water relaxation rates. We are currently measuring cross relaxation effects in other hydrated biological systems and evaluating its consequences for a published analysis[9] of the relaxation behaviour in hydrated collagen.

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Effect of NO photolysis on NO_y mixing ratios

IT has been suggested that the only significant sink for odd nitrogen in the stratosphere is through transport of HNO_3 to the troposphere[1]. As a result, if NO were to be injected in the middle or lower stratosphere, the change in odd nitrogen, NO_y ($NO_y \equiv NO + NO_2 + HNO_3 + N$), mixing ratio above the height of injection would be nearly constant. The photolysis of nitric oxide, however, and the subsequent reactions of nitrogen atom with nitric oxide act as a sink for odd nitrogens in the upper stratosphere. This mechanism has long been recognised as important to mesospheric chemistry[2,3], and has also been included in many of the stratospheric models[4–7]. Its role in the stratospheric NO_y budget, however, has not been analysed. This paper reports an investigation on the importance of this net NO_y sink by means of a one-dimensional transport-kinetics model[8].

In this model the reactions

$$\mathrm{NO} \xrightarrow{h\nu} \mathrm{N} + \mathrm{O}$$
$$\mathrm{N} + \mathrm{NO} \rightarrow \mathrm{N_2} + \mathrm{O}$$
$$\mathrm{N} + \mathrm{NO_2} \rightarrow \mathrm{N_2O} + \mathrm{O}$$

provide a chemical sink for odd nitrogen in the upper stratosphere. We studied the effect of NO photolysis on NO_y mixing using a parameterisation of NO photolysis as shown by Cieslik and Nicolet[9] and the chemical reactions used by Duewer *et al.*[10]. We found that inclusion of the sink would not result in a constant change in odd nitrogen mixing ratio above the height of a NO_x injection. This sink has a significant effect on model predicted effects of chlorofluoromethanes.

We computed an ambient NO_y mixing ratio and a perturbed NO_y mixing ratio due to an injection of NO at a rate of 2,000 molecules $\mathrm{cm}^{-3}\,\mathrm{s}^{-1}$ in a 1-km thick layer centred at 17 km using our standard chemistry (with the rate constant for $OH + HO_2 \rightarrow H_2O + O_2$ equal to $2 \times 10^{-11}\ \mathrm{cm}^3\,\mathrm{s}^{-1}$). We then repeated the calculation with the rates of the NO photolysis sink set equal to zero.

Both of the above sets of calculations were repeated for the Hunten[11] eddy diffusion profile and for the Crutzen[12] eddy diffusion profile.

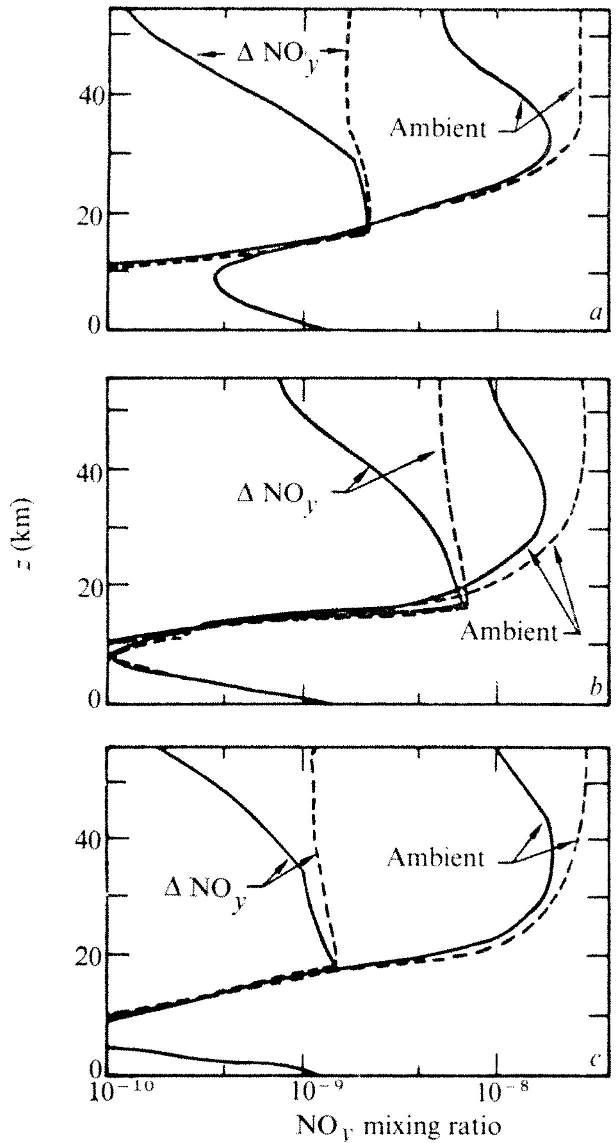

Fig. 1 Plots of computed ambient NO_y mixing ratios (ambient) and changes in NO_y mixing ratio after a 17-km NO_x injection (ΔNO_y) as described in text. ———, normal model; — — —, model with the NO photolysis sink suppressed. *a*, Chang[8] K_z profile; *b*, Hunten[11] K_z profile; *c*, Crutzen[12] K_z profile.

The NO_y mixing ratios resulting from the above calculations are given in Fig. 1. A comparison of the results with and without the NO photolysis sink implies that this sink for NO_y is responsible for both decreasing (NO_y) at all altitudes and for a decrease in NO_y mixing ratio with altitude above about 30 km.

Note that for the perturbed cases the changes in mixing ratio are not constant with altitude even when the photolysis sink (the only chemical sink for NO_y in the model) is removed. This is a result of a change in the net rate of the 'natural' production of NO_y from the reaction

$$N_2O + O(^1D) \rightarrow 2NO$$

This source term changes because the ozone concentration changes. The ozone reduction leads to a decrease in $O(^1D)$ concentrations at high altitudes where the proportional change in solar flux is less than the change in ozone concentration, and an increase in $O(^1D)$ at low altitudes, where the change in solar flux density more than compensates for the change in ozone. The changes in N_2O (because of altered solar flux) are relatively minor. The net change in the production of NO_y from $O(^1D) + N_2O$ in the model with NO photolysis suppressed, is given in Fig. 2 for the three diffusion profiles.

The net changes in ozone column for the above calculations are given in Table 1. For ozone reductions, note that the rate coefficients used in the current calculations are the recommended values from Hampson and Garvin[13], with 2×10^{-11} cm^3 s^{-1} for $OH + HO_2$. Thus, there are numerous minor changes and one major change in the rate coefficients used here compared to those used to generate the CIAP findings[11]. Although omission of the N atom sink for NO_y makes a very substantial difference in the NO_y mixing ratio above 31 km, it causes only a minor increase in the percentage ozone perturbation for NO_x injections. This results because both the ambient and perturbed NO_y mixing ratios are reduced by the sink. The ozone perturbation is more strongly affected for the Hunten profile, which was used in the formula developed by NAS[11], than for the other diffusion profiles considered.

The effect of the N atom reactions on the O_3 response of the model to a ClO_x injection is opposite in sign and larger than their effect on the O_3 response to an NO injection. We find that, when the N atom sink is removed from our model, the stratosphere is substantially less sensitive to chlorofluorocarbons than when it is included. A model that computed a 0.51% reduction in ozone due to chlorofluorocarbons in 1976 using our full chemistry computed only a 0.30% ozone reduction in 1976 when NO photolysis was neglected. This effect occurs because the reaction

$$NO + ClO \rightarrow NO_2 + Cl$$

reduces the efficiency of both the chlorine and NO_x ozone destruction cycles, and the effect of this reaction is especially dependent on the NO_x mixing ratio above 35 km.

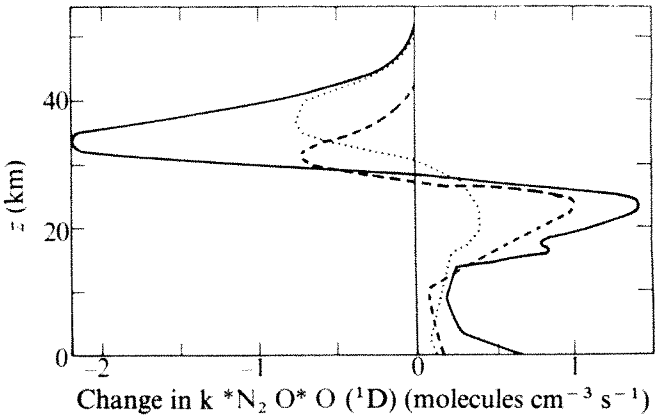

Fig. 2 Change in the odd nitrogen production rate in models with the NO photolysis sink suppressed. ———, Hunten (1974) K_z profile; — — —, Chang[8] K_z profile; ····, Crutzen (1975) K_z profile.

Table 1 Ozone columns (molecules cm^{-2}) and percentage deviations

	K_z (Chang)	K_z (Hunten)	K_z (Crutzen)
Normal, ambient O_3	7.4334×10^{18}	7.4627×10^{18}	7.4437×10^{18}
Normal, 17-km NO source	7.2920×10^{18}	6.9644×10^{18}	7.3459×10^{18}
ΔO_3 (%)	−1.75	−6.68	−1.31
No N atom ambient	7.2752×10^{18}	7.148×10^{18}	7.1165×10^{18}
No N 17-km NO source	7.1467×10^{18}	6.630×10^{18}	7.0195×10^{18}
ΔO_3 (%)	−1.77	−7.25	−1.36

Although models of the effects of NO_x injections on the stratosphere are not very sensitive to the NO photolysis sink for NO_x, inclusion of this sink is quite important for models of the effects of chlorofluorocarbons in the stratosphere. Indeed, the concentration of NO above 35 km is an important and poorly validated quantity in current models.

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Quantitative effects of sediment mixing on stratigraphy and biogeochemistry: a signal theory approach

SEDIMENT mixing by benthic fauna greatly affects the detailed historical record of the environment preserved in the sediments[1]. Mechanical stirring is a major transport mechanism affecting fluxes of solid material and dissolved chemicals between sediments and the water column[2]. Therefore, chemical and microbiological reaction rates in sediments are coupled to the rate at which substrate and product are transported to and from the site of reaction by bioturbation[3]. In order to understand better these processes, models which allow quantitative prediction of sediment mixing effects on inert and reactive sedimentary constituents are desirable.

The complexity of mixing events by benthic infauna has generally resulted in neglect of the environmental effects of mixing. Previous models have described mixing by a parametric 'diffusion coefficient'[4] or 'mixing depth'[5–8], but such models have been applicable only under restricted assumptions of steady-state sedimentation and of constant and invariant mixing.

I have developed a general signal theory based model of bioturbation that does not require assumptions of temporal or spatial steady state, or of constant mixing to an invariant depth (Goreau, unpublished). This method allows calculation of the effect of any series of time-varying mixing events on any time-varying input to the sediment. It thus allows, when we have sufficient knowledge of the mixing history, the recovery of some original input information from sediments that have had their stratigraphic records mixed by bioturbation by comparison of actual profiles with model profiles based on realistic values of the relevant parameters.

As mixing moves material to shallower and deeper depths in sediments, corresponding to past and future inputs, there is no unique age at each depth, but rather a mixture of material of different ages. It is useful to define a 'mixing function' $F(x)$, the depth profile of some unit quantity of inert material that has been introduced to the sediment surface at a discrete time and then been mixed and buried. Figure 1*b* shows the 'mixing functions' that result from the mixing and burial of three separate historical inputs to the sediment. If there were no mixing these would be found at depths X_0, X_1 and X_2. If we consider the profile that would result if some time-varying input was delivered to the sediments in the absence of mixing, $I(x)$ (Fig. 1*a*), then it can be shown that the mixed profile $O(x)$ (Fig. 1*c*) that results from the action of mixing on the input can be calculated as a convolution integral of input $I(x)$ and the mixing function $F(x)$:

$$O(x) = \int_{\infty}^{0} I(x')\,F(x-x')\,\mathrm{d}x' \qquad (1)$$

where x' is a dummy variable and mixing is represented as spatial filtering of the undisturbed input by the mixing function $F(x, x')$. The mixing process acts as a low pass frequency filter, allowing slow changes in input to be recorded in the sediment while attenuating rapid changes. Fine structure of the stratigraphic record is smeared out in a predictable manner, and recent events are recorded deeper in the sediment than they would be in the absence of mixing (Fig. 1*d*). As a result, estimates of sedimentation rates and palaeo-oceanographic changes derived from penetration depths of such recent inputs as the bomb-produced radionuclides ^{14}C and ^{137}Cs are often too high. The true value can be found if $F(x)$ is known.

$F(x)$ can be determined from measurements of $O(x)$, the natural mixed profile of a non-reactive constituent with known inputs, and measured directly when that input is a spike such as thin ash layers, microtektites, or rapid microfossil changes. When $F(x)$ is known at a particular site, it is applicable to other inert inputs, and can be used in conjunction with measured mixed profiles to deconvolve the effects of mixing by inversion of equation (1) to derive the original input history.

A similar mixing function is derived for pore water, $F_w(x)$, the volume of pore water exchanged with overlying seawater per unit area per unit time, as a function of depth (Fig. 1*e*). If the concentration of dissolved constituent with depth is $C(x)$ (Fig. 1*f*), and C_w in seawater, then the flux of the constituent across the sediment–water interface is shown to be

$$\text{Flux (g cm}^{-2}\,\text{s}^{-1}) = \int_{0}^{x_{\max}} F_w(x)\,(C(x) - C_w)\,\mathrm{d}x \qquad (2)$$

When $C(x)$ exceeds C_w the flux will be from sediment to the water column, and when $C(x)$ is less than C_w the net flux will be into the sediments. This expression contrasts with Fickian diffusion terms used to estimate fluxes in previous work[9], since bioturbation is much faster than molecular diffusion. Ions transported across the interface have their source or sink throughout the mixed zone of sediment, and are not just passively driven by diffusion along pore water concentration gradients at the interface, as assumed in diffusive flux calculations. Measured values of $F_w(x)$ may substantially modify estimated sediment–water geochemical fluxes.

Reactive inputs, consumed or produced in sediment, will have different depth profiles than inert inputs. The difference between mixed profiles resulting from similar reactive and conservative inputs is a measure of net consumption or production of inputs with depth. By interpreting carefully controlled laboratory experiments in conjunction with the present approach, I believe it possible to estimate means and ranges of net reaction rates as a function of depth in naturally mixed sediments to obtain quantitative measures of geochemical and microbially mediated reaction rates in sediment. These rates should be quite different from those estimated in the absence of mixing, since redox potential dependent reactions are affected by the rate of oxygen

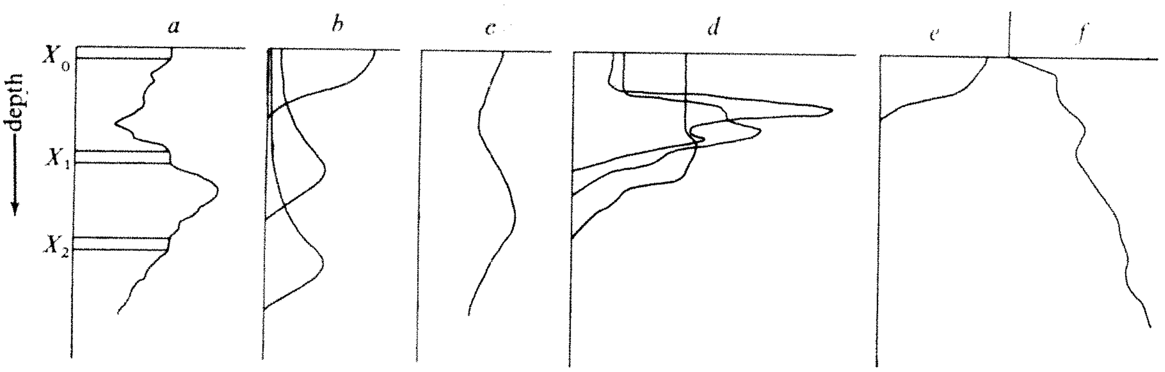

Fig. 1 *a*, An undisturbed time varying sedimentary input, $I(x)$, that would be observed in the complete absence of mixing. *b*, Mixing functions showing the depth profiles caused by the mixing and burial of three successive inputs, $I(x)$, $I(x_1)$ and $I(x_2)$. *c*, $O(x)$, the mixed output obtained by operation of the mixing function on $I(x)$. The mixed output is derived from the time-varying input and mixing function through the finite difference form of equation (1): $O(x) = \Sigma_i I(x_i) F(x, x_i)$. *d*, Calculated effects of uniform mixing to 1, 2 and 4 relative depth units on the fallout inputs of the bomb-produced radionuclide ^{137}Cs. (Increased depth of mixing is seen to push the ^{137}Cs maximum deeper into the sediment and obliterate fine scale information in the mixed profile.) The calculated profiles are similar to those measured by Pennington *et al.*[10]. This choice of mixing functions produces results similar to those of Robbins and Edgington[11]. *e*, $F_w(x)$, the volume of pore water exchanged with overlying water per unit area per unit time, as a function of depth, due to bioturbational mixing of sediment and faunal water pumping. *f*, A typical depth profile of a dissolved nutrient such as ammonia or phosphate in pore water. The flux of the dissolved constituent across the sediment–water interface is given by equation (2).

transport into sediment. Bioturbation affects oxygen fluxes, the Eh microhabitat, and ecological depth zonation of the microfauna that mediate such environmentally important processes as aerobic heterotrophic decomposition, sulphate and nitrate reduction, and ammonia and methane generation.

This work shows that many stratigraphic, geochemical and microbiological problems in recent sediment can be approached through measurable 'mixing functions' characterising the real-world dynamic effect of mixing in a quantitative and predicitively useful manner. Further development of the numerical model with applications to the spatial and frequency response of the historical record, detailed stratigraphic records of bomb-produced radionuclides, the deconvolution of recent pollutant trace-metal sediment profiles, sediment–water fluxes, temporal and spatial variation, and the estimation of sediment geochemical and microbiological reaction rates will be published elsewhere.

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Evidence of major sulphate evaporite deposits in the Proterozoic McArthur Group, Northern Territory, Australia

WE report the occurrence of extensive sulphate evaporite relicts from the McArthur Group (1,600–1,400 Myr old), Northern Territory (16° 30′ S: 135° 30′ E approx), Australia. The McArthur Group comprises a sequence of carbonates, shales, siltstones and sandstones, with a minor volcanic component, of mid-Proterozoic sediments and includes the huge H.Y.C. stratiform Pb–Zn–Ag deposit[1].

Some models for the early evolution of the Earth's hydrosphere[2] postulate that sulphate evaporite minerals were not deposited before late Proterozoic times (~800 Myr ago)[3–7]. However, our work, and other evidence[8–11] indicates that these evaporite minerals do indeed occur in older rocks.

Pseudomorphs after halite and gypsum were first reported from the Group in the 1960s[12–14]. Our present studies show that these and other evaporite relicts are much more widespread and significant than was originally thought. Most of the evaporite relicts are in the form of carbonate pseudomorphs after a variety of morphologies of gypsum and anhydrite crystals, chert pseudomorphs after anhydrite nodules (=the 'botryoidal quartz' of ref. 5), halite casts, and microscopic remnants of original, unaltered sulphate minerals. The characteristics of each of these evaporite relicts are described and their distribution within the McArthur Group is shown in Table 1.

The most abundant evaporite relicts in the McArthur Group are carbonate pseudomorphs after discoidal and euhedral to subhedral gypsum and anhydrite crystals (Fig. 1*a*). The size of the pseudomorphs is variable. The discoidal crystals range in length from less than 1 cm to about 15 cm. They are lensoid in cross section, with length to breadth ratios varying from about 2 : 1 to 15 : 1. The euhedral pseudomorphs are less common than the discoidal ones and range in length from about 5 mm to 3 cm. We have found discoidal pseudomorphs in quartz arenites, and they are very abundant in a number of carbonate lithologies.

In quartz arenites, the pseudomorphs are disseminated and randomly oriented, and they contain numerous small inclusions of quartz and dolomite The distribution of the pseudomorphs in the carbonate rocks, however, varies from a few randomly oriented and disseminated specimens, to whole beds of pseudomorphs (originally mapped as sideritic marbles[12]), ranging from 10 cm to 45 cm thick. In the massive beds, discontinuous wisps of chert characteristically delineate algal mats, domal stromatolites and *Conophyton*. In the carbonate rocks, pseudomorphs cross cut sedimentary features such as bedding and laminated algal mats, suggesting that the original sulphate minerals crystallised in the host sediments during diagenesis. The pseudomorphs may be either single crystals or mosaics of anhedral grains displaying a polygonal texture. The grains vary in diameter from 50 to 100 μm, and are generally larger than the grain size of the host sediment. As well as carbonate, the pseudomorphs frequently contain traces of disseminated pyrite, and occasional blebs of chalcedonic quartz. Electron probe microanalyses of the carbonate minerals show that they are iron-rich phases such as ferroan dolomite, ferroan magnesite (bruenerite) and magnesian siderite (pistomesite), whereas the host carbonate is usually less iron-rich dolomite or magnesite. Some pseudomorphs are compositionally zoned with respect to their iron content. In some of the pseudomorphs, we have observed tiny, highly birefringent inclusions, reaching a maximum size of 10–20 μm, and have identified them as anhydrite, using the petrological microscope. Our identification was confirmed by electron probe microanalysis. Using these techniques, we have also identified nodules of anhydrite averaging 2–3 mm in diameter from a silicified algal mat horizon in a massive pseudomorph bed in the Amelia Dolomite.

The discoidal pseudomorphs are identical in habit with the gypsum which grows interstitially in Recent and ancient sabkha and intertidal environments[15–18]. Both have curved faces and both are frequently flattened in a plane perpendicular to the *c* crystallographic axis. Others, however, have spear shaped or serrated terminations that possibly reflect repeated twinning of the original crystals (Fig. 1*b*). Such forms are also characteristic of anhydrite crystals that are found in Recent and ancient marginal sabkhas[17–19].

Apart from the discoidal and euhedral pseudomorphs described, the McArthur Group also contains acicular carbonate pseudomorphs. These were tentatively identified as being dolomite pseudomorphs after aragonite[20], and were believed to be restricted to the Coxco Dolomite Member of the Teena Dolomite (Table 1). Our studies, however, show that as well as the Coxco Dolomite occurrence, acicular pseudomorphs are also present in the Emmerugga Dolomite. The needles are six-sided, and their interfacial angles are the same as those of gypsum crystals elongated parallel to the *c* crystallographic axis (see Fig. 2). The acicular pseudomorphs vary from 2 to 10 cm in length, and from 1 to 5 mm in diameter, and they occur in radiating clusters. In thin section, the needles are seen to be composed of mosaics of anhedral carbonate grains with an average diameter of 100 μm. The pseudomorphs are zoned, having rims of clear dolomite, with cores of dark dolomite containing abundant fine-grained inclusions of what appear to be clay minerals. The carbonate mineral in the pseudomorphs is ferroan dolomite identical in composition with the host dolomite.

The acicular pseudomorphs are identical in habit to a variety of gypsum forming in shallow brine pools on the sabkhas of the Trucial Coast (D. Kinsman, private communication). According to Kinsman, the root of each cluster is situated at the sediment–water interface. The larger acicular gypsum needles are often hollow and partly filled with fine-grained detritus. We therefore interpret the zoning of the McArthur acicular pseudomorphs to have been formed by similar trapping of fine-grained detritus in once hollow gypsum needles, although they may have been

Fig. 1 *a*, Distribution of carbonate pseudomorphs in carbonate beds. (Scale bar : 0.5 cm.) *b*, Evaporite pseudomorphs with serrated terminations. (Scale bar : 0.5 cm.) *c*, Botryoidal quartz nodule. *d*, Thin section of botryoidal quartz, showing lutecite crystals. (Scale bar : 0.5 mm.)

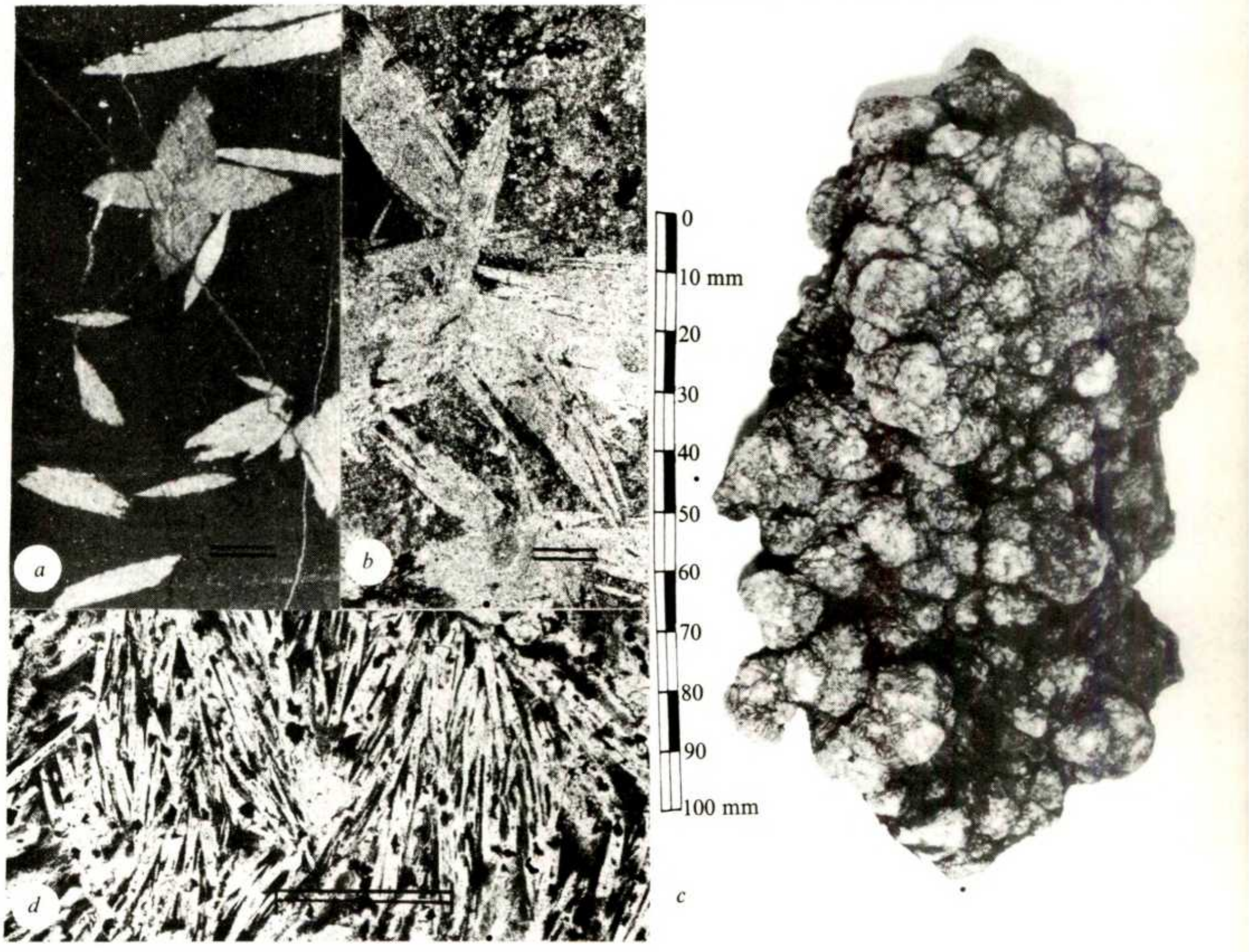

Table 1 Regional stratigraphy and distribution of evaporites, in the McArthur Group. H, halite casts; LD, euhedral and discoidal gypsum and/or anhydrite pseudomorphs; N, acicular gypsum pseudomorphs; C, cauliflower chert nodules

	Formation	Main lithologies	Thickness (m)	Evaporite pseudomorphs
	Stott Formation	Massive and stromatolitic dolomite; dolomitic shale	750	LD
	Smythe Sandstone	Quartz sandstone; chert conglomerate	0–180	
Batten Sub-group	Looking Glass Formation	Chert; chert breccia; minor dolomite	25–230	
	Stretton Sandstone	Flaggy quartz sandstone	30–245	
	Yalco Formation	Chert; chert breccia; minor dolomite	130	
	Lynott Formation	Dolomitic siltstone; chert; chert breccia; minor dolomite	210–760	LD, C (Donnegan Member)
Umbolooga Sub-group	Reward Dolomite	Dolomite with chert pellets; dolomitic siltstone	50–300	
	Barney Creek Formation	Pyritic, carbonaceous, dolomitic shale; dolomite; dolomite breccia; H.Y.C. deposit	0–490	
	Teena Dolomite	Massive laminated and stromatolitic dolomite	57	H, N (Coxco Dolomite Member)
	Emmerugga Dolomite	Massive laminated and stromatolitic dolomite	300	H, LD, N
	Tooganinie Formation	Stromatolitic dolomite; dolomitic shale; quartz arenite, dolomitic siltstone	800	H, LD
	Tatoola Sandstone	Quartz arenite; dolomitic siltstone	140	LD
	Amelia Dolomite	Laminated and stromatolitic dolomite, massive gypsum pseudomorphs in beds	90–240	H, LD, C
	Mallapunyah Formation	Purple dolomitic siltstone; minor dolomite; quartz arenite	30–750	H, LD, C

formed by a mechanism similar to that suggested by Kastner[21].

'Cauliflower' chert nodules[23] also occur in the McArthur Group, and are particularly abundant in the Mallapunyah Formation, and in the Donnegan Member of the Lynott Formation (Table 1). The nodules are roughly circular in plan and are flattened parallel to the bedding. They vary from about 1 to 100 cm in diameter, and from 1 to 30 cm in height. The upper surfaces of the nodules are irregular and botryoidal (Fig. 1*c*). The lower surfaces are similar but more flattened. The outer surfaces have a well developed mesh-like structure of interlocking chert blades. In thin section three zones can be recognised. The outer zone consists of a mesh of lutecite fibres[22] set in a matrix of brown microcrystalline quartz (Fig. 1*d*). In some cases, the fibres display a distinct, sub-parallel lineation, and they contain highly birefringent inclusions which we have identified as anhydrite. This outer zone grades inwards to a zone of macrocrystalline quartz that contains minor amounts of dolomite and barite. The innermost zone of the nodules consists of coarsely crystalline dolomite and barite, together with trace amounts of fluorite. These nodules are remarkably similar to the chert nodules from the Fort Payne and 'Warsaw' Formations of Tennessee which Chowns and Elkins[23] demonstrated to be replacements of anhydrite nodules identical with those described by Kinsman from the sabkha environments of the Persian Gulf[19].

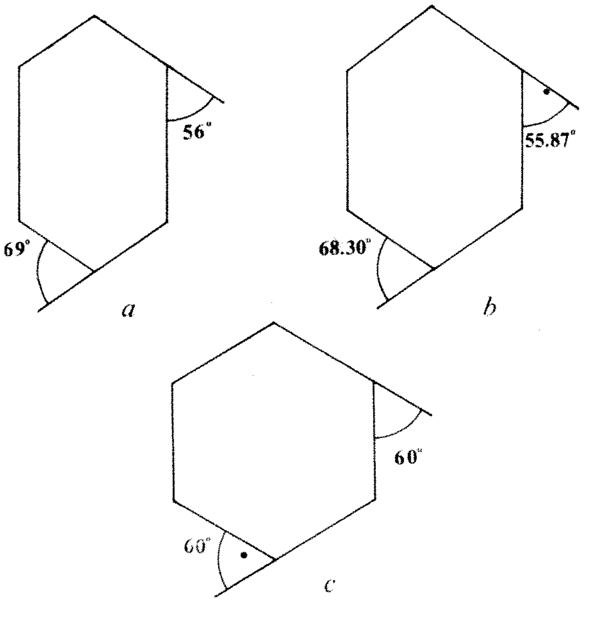

Fig. 2 Interfacial angles of pseudomorphs in radiating clusters (*a*) compared with interfacial angles of gypsum (*b*) and aragonite (*c*).

Pseudomorphs after halite are common throughout the McArthur Group with the highest concentration in red beds and some dolomitic siltstone sequences (Table 1). Brown and Plumb[24] describe the characteristics of the halite casts in the Group, and the present studies have produced little new information. The halite casts which we have seen up to now, all appear to have formed by almost complete evaporation in shallow marine environments, and probably represent ephemeral salt crusts. Pseudomorphs after halite coexisting with either gypsum or anhydrite are extremely rare. This general lack of association of halite and calcium sulphate minerals probably results from the dissolution of previously deposited halite during surface flooding, as it does in Recent marginal sabkhas[17,18].

While we cannot determine precisely the amount of evaporite material because of poor outcrop in the McArthur River area, our studies show that evaporitic sediments occur over an area of at least 5,000 km^2 in the Batten Trough alone[25]. In some sections of the particularly evaporitic formations, for example, the Amelia Dolomite, up to 40% of the measured sections are composed of evaporite relicts. Therefore, not only do our studies show that a variety of evaporite relicts occur in the McArthur Group, but that they occur in vast amounts.

Although our work is still at an early stage, the results obtained so far lead us to several important conclusions. First, we conclude that significant parts of the McArthur Group were deposited in a marginal sabkha environment. Many of the evaporite sequences of the McArthur Group are remarkably similar to the Recent sediments of the Trucial Coast. This resemblance provides the basis for new insights into the sedimentary environments represented in the McArthur Group, the habitats of the microfossils which abound in many of the formations[26–28], and suggest possible physiographic configurations and climatic regimes. Second, the McArthur Group evaporites which are similar to Recent coastal sabkha deposits in terms of chemistry, crystallographic habit etc., are probably the products of brines with essentially the same compositions. Third, the Persian Gulf sabkha model is not applicable to all of the McArthur Group evaporites, some of which appear to have stronger resemblances to the Marion Lake, Yorke Peninsula (South Australia) lacustrine gypsum deposits[29]. Fourthly, our results show conclusively that the giant H.Y.C. stratiform Pb–Zn–Ag deposit at McArthur River is yet another example of the widespread association of stratiform base metal sulphide deposits with sulphate evaporitic sediments[30–32]. The location of the H.Y.C. deposit within a large evaporite bearing basin raises the interesting possibility that this and related deposits formed from metal-rich brines of the kind forming today in evaporite-bearing basins[33], rather than from volcanically derived hydrothermal solutions[34].

Finally, we maintain that the evidence presented here and elsewhere[4] unequivocally shows the widespread occurrence of large volumes of sulphate evaporites in mid-Proterozoic times. The assertion that there was no significant deposition of such evaporites before ~800 Myr ago[2] is manifestly in error. Consequently, the models of crustal, hydrospheric and atmospheric evolution[2] based on this contention require considerable revision and modification.

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Discovery of amphibians in the Namurian (Upper Carboniferous) of Fife

CARBONIFEROUS amphibians are very rare as fossils and almost all major finds of British specimens were made in the nineteenth century. Nevertheless, knowledge of Carboniferous tetrapods, both amphibians and reptiles, has expanded considerably in recent years[1,2]. All are from Europe or North America and most are from horizons equivalent to or later than the British Coal Measures (Westphalian and Stephanian, respectively). Far fewer amphibians are known from the Lower Carboniferous and knowledge of the Namurian tetrapod fauna, between the Lower Carboniferous and the Coal Measures, is even more meagre. With the exception of a single incomplete anthracosaur amphibian from the Namurian of the Ruhr, Germany[3], pre-Coal Measure amphibia are known from only three areas (1) West Virginia and south-western Pennsylvania in the United States[4,5]; (2) Nova Scotia, Canada[6], and (3) the Edinburgh area and southern Fife in Scotland[7,8]. In November 1974, however, fossil amphibian and fish remains were discovered at the Dora Opencast Site (area C), south-east of Cowdenbeath, Fife (Fig. 1), in Carboniferous strata of Namurian age. The richly fossiliferous bone bed at Cowdenbeath formed a localised patch in a seatrock (rooty, muddy siltstone), which lies beneath a coal seam below the Lochgelly Blackband Ironstone. The Lochgelly Blackband Ironstone itself has also produced a fauna with amphibian remains in the Dora site, while a few specimens have been recovered from strata up to 6 m lower in the sequence.

All these strata belong to the Limestone Coal Group which forms the lowest subdivision of the Namurian Series (lower Namurian A) in Scotland. In the Dora area this subdivision is about 330 m thick. A measured section (Fig. 2) at the locality indicates the position of the bone bed about 62 m beneath the base of the Index Limestone at the top of the Limestone Coal Group. Currie[9] took the base of the Index Limestone as the junction between the top of the Pendleian (E1) and the base of the Arnsbergian (E2) stages, a conclusion which is supported by Wilson (ref. 10, Fig. 1).

The bone bed was originally exposed as a section some 30 m long, but thanks to the generosity of the Murphy Group, contractors to the National Coal Board, the considerable overburden was removed to expose the upper surface of the bone bed, which dipped gently north-westwards. The bed was cut off by a fault at the south-west end and divided by two small faults into three areas. Three 1.25-inch (31-mm) diameter cores were taken from the surface of the bed to a maximum depth of 5 m, by K. Hall and S. Creany

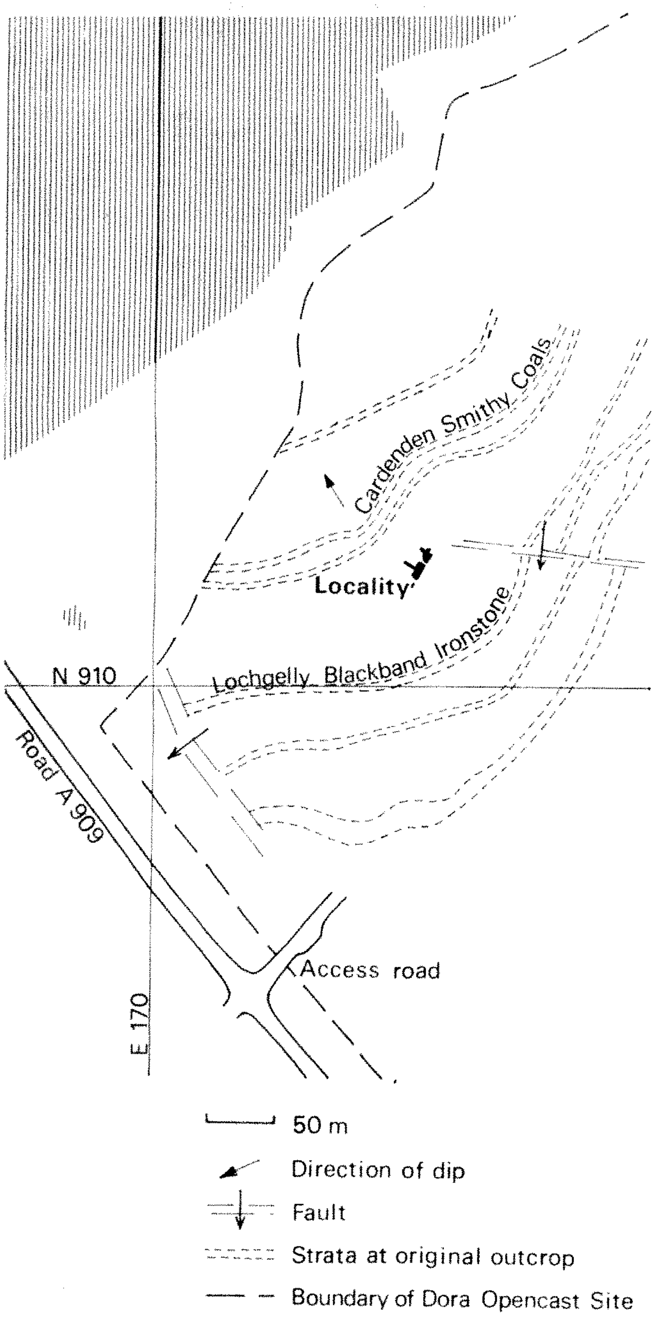


Fig. 1 Position of the fossil amphibian locality in Dora Opencast Coal Site, south-east of Cowdenbeath, Fife (NT 172911). From National Coal Board Opencast Executive Scottish Region, Drawing No. 01/6028.

of the Organic Geochemistry Unit, University of Newcastle upon Tyne. After gridding into 0.33-mm squares to preserve relationships between associated remains, the richest part of the bone bed was collected by hand. This material, together with transects extending outside the richly fossiliferous area, is stored in the University of Newcastle upon Tyne, in the Royal Scottish Museum and by S. P. Wood. After a delay from October to December 1975 to facilitate collection, opencast operations have removed all the surrounding and underlying strata.

Several specimens have already been developed from the bone bed. Many of these are isolated skeletal elements of sharks and of representatives of all the major groups of bony fish known from the Carboniferous. These include acanthodians, actinopterygians, dipnoans (lungfish) and crossopterygians, the group from which the tetrapods are agreed to have descended. The acanthodian fish *Gyracanthus* is by far the commonest form, represented in nearly

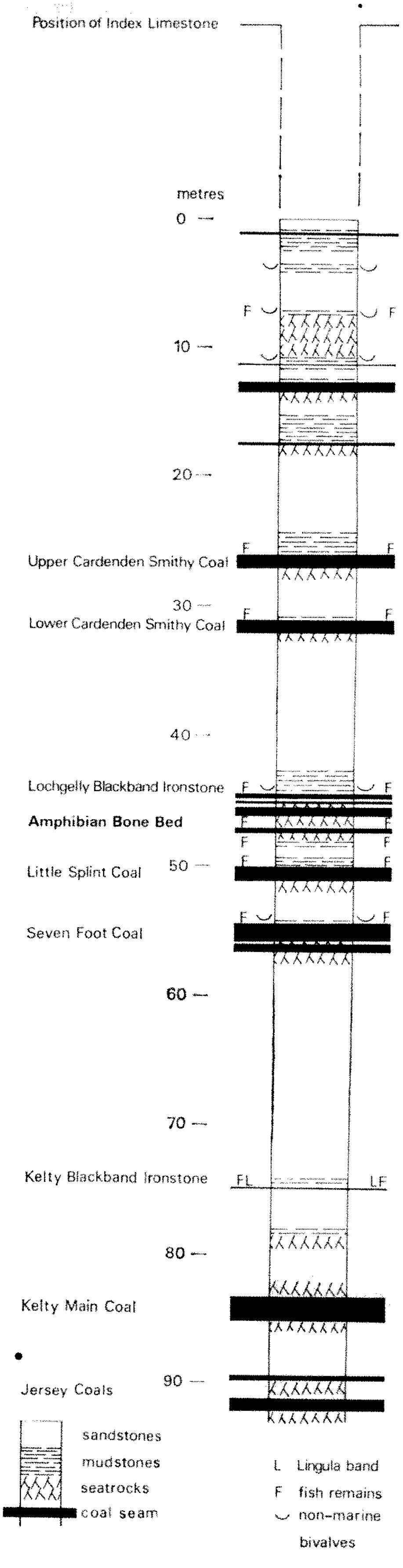


Fig. 2 Composite measured section in Dora Opencast Site (NCB), areas *C* and *D* (Institute of Geological Sciences registered numbers NT 19 SE/534 and 537).

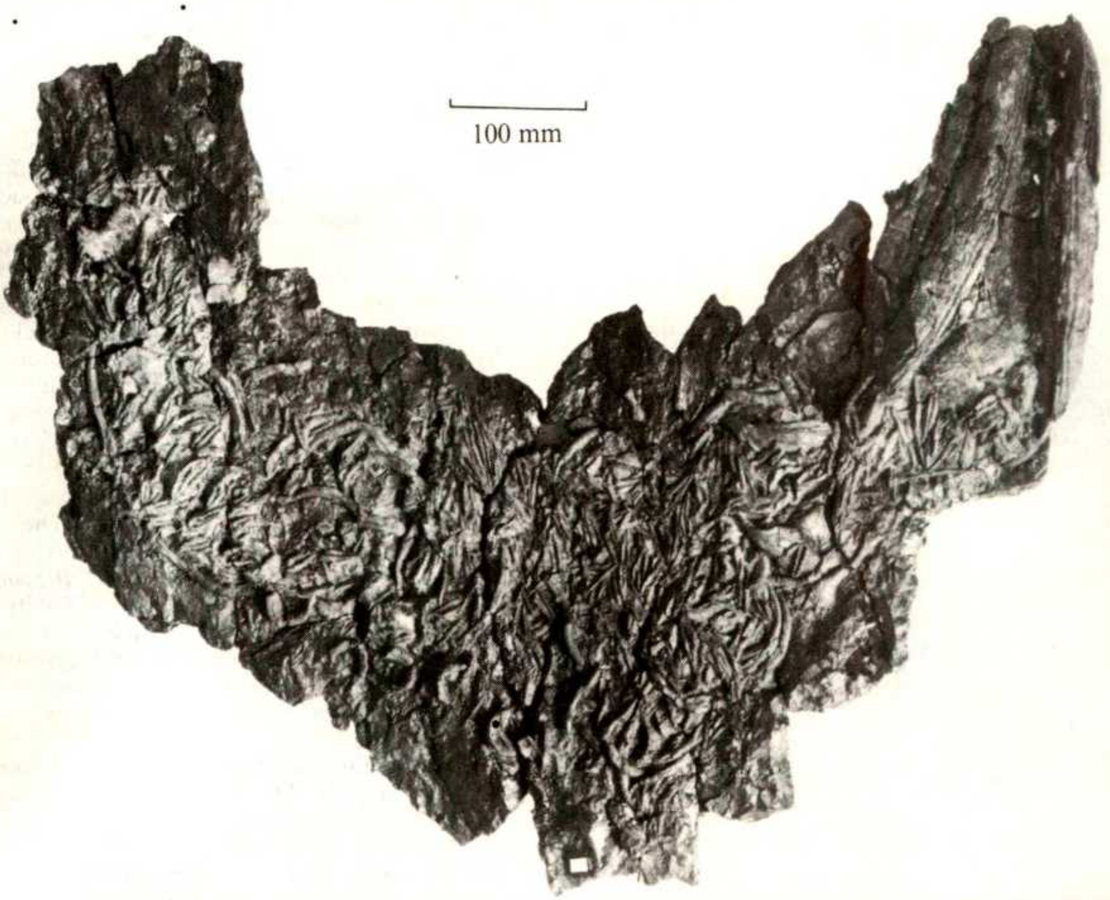


Fig. 3 Skeleton of a new anthracosaur amphibian (BM(NH)R 10,000) in ventral view.

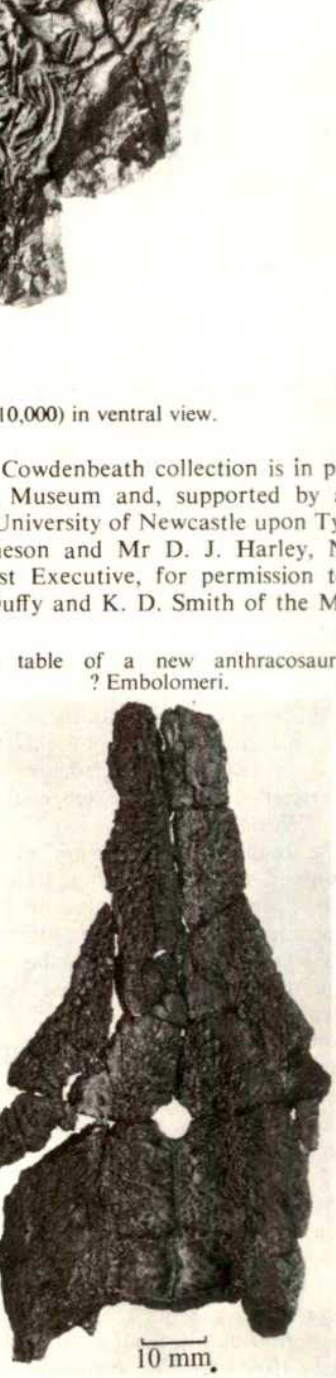
every 0.33-m block of matrix by at least one spine, whereas the actinopterygians are represented only by a single specimen showing palaeoniscid scales. The skull bones of rhizodontiform crossopterygians will give important information on this poorly known group. The fish fauna of the Lochgelly Blackband Ironstone appears to be similar to that of the bone bed but includes six determinable actinopterygian species.

The amphibian specimens include representatives of all three major Palaeozoic groups, the Anthracosauria, Temnospondyli and Lepospondyli. By far the most important specimen so far developed is the almost complete but tail-less specimen of a new crocodile-like anthracosaur amphibian, now registered in the British Museum (Natural History) (Fig. 3). Skull remains of a second anthracosaur are probably to be referred to a primitive Lower Carboniferous species, *Eoherpeton watsoni* Panchen[2] and a third anthracosaur, as yet undescribed, is represented by the skull table and interorbital region (Fig. 4). Temnospondyls include a jaw ramus of the aberrant loxommatid *Spathicephalus mirus* Watson[7], previously known only from a similar horizon at Loanhead near Edinburgh, and appendicular bones probably to be referred to the primitive long-bodied Colosteidae[2]. The small lepospondyl amphibia are represented by isolated postcranial remains.

The Carboniferous was the period of the first major adaptive radiation of tetrapod vertebrates and the earliest reptile known is from the early Coal Measures (Westphalian B) of Nova Scotia[11]. The Cowdenbeath find could extend reptile history down into the Namurian. It also has the potential to vastly increase knowledge of the early Carboniferous fauna beyond the small number of species and individuals from other early Carboniferous sites. Furthermore, the Namurian fauna is particularly important in forging the evolutionary link between the few primitive amphibia known from the Lower Carboniferous and the more advanced and diverse fauna of the Coal Measures.

Study of the Cowdenbeath collection is in progress at the Royal Scottish Museum and, supported by a grant from NERC, in the University of Newcastle upon Tyne. We thank Dr F. J. Matheson and Mr D. J. Harley, National Coal Board, Opencast Executive, for permission to collect and Messrs A. P. Duffy and K. D. Smith of the Murphy Group

Fig. 4 Skull table of a new anthracosaur amphibian, ? Embolomeri.



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Earliest known *Hipparion* from Holarctica

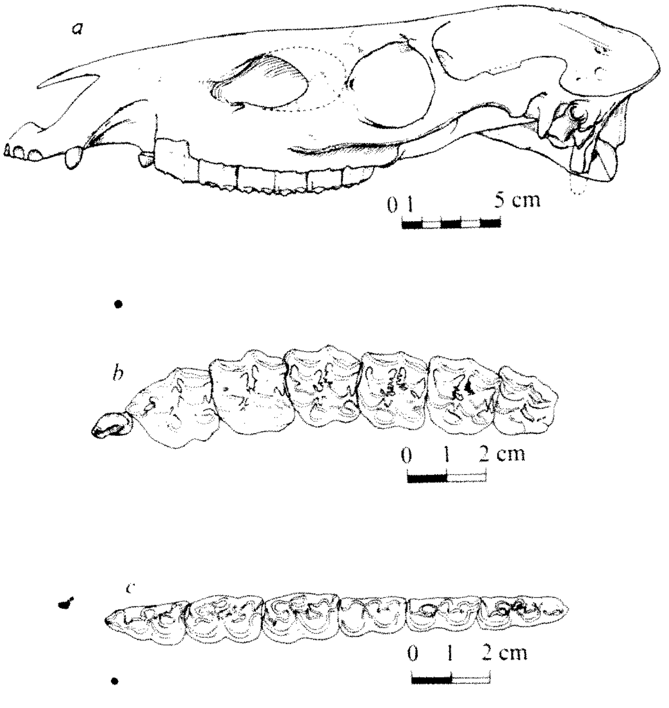
The three-toed horse *Hipparion* was ubiquitous in terrestrial mammalian faunas throughout Holarctica during parts of the Miocene. This report describes *Hipparion* from the early Barstovian Fleming Formation of the Texas Gulf Coastal Plain. These specimens are assigned to *Hipparion* because of dental characters such as the diagnostic presence of isolated protocones in the upper molars. This *Hipparion* is also characterised by a well-developed and anteriorly-rimmed preorbital fossa similar to the condition seen in some Old World forms. Because of this earliest known occurrence, the widely used *Hipparion* datum concept should be restricted to some palaeogeographically finite area in the Old World.

We prefer the designation '*Hipparion*' in the broad sense because we contend that, in common usage, it represents a 'form genus' consisting of Miocene–Pliocene horses with isolated protocones in the upper molars and tridactyl feet, that is, *Hipparion* is not used in the strict taxonomic sense. Because of its great abundance both geologically and geographically, this horse has played a significant role in the biochronology of this time interval, with Eurasian Neogene mammalian assemblages often being termed *Hipparion* faunas. The first appearance of *Hipparion* has been used as a geologically synchronous time plane, also termed 'datum', much in the same way as microfossils have been used in marine sequences. Based on a combination of different geochronological techniques, the *Hipparion* datum has been determined to be 12.5 Myr in the western Old World[1,2], 12.8±1.03 Myr in northwestern Africa[3], and 12–9 Myr in Sub-Saharan Africa[4]. In the well known mammal-bearing Siwalik sequence of the Sub-Himalayan region, the *Hipparion* datum is above the base of the Nagri Sandstone and *Hipparion* is common in the middle Siwaliks[5–7]. Precise determinations for this datum are not available. *Hipparion* is also abundant from parts of the Nearctic Miocene. Until now, the first occurrence of this horse in the New World has been thought to be of early Clarendonian age.

In the collection at the American Museum of Natural History, New York, are several skulls and dentitions of *Hipparion* from Trinity River Pit 1, early Barstovian age (medial Miocene), Fleming Formation, San Jacinto County, Texas Gulf Coastal Plain (Fig. 1). There is no direct geochronological control for this locality. Based on correlation to other early Barstovian assemblages with radiometric age determinations, the Trinity River Pit 1 seems to be significantly older than the Old World localities in which *Hipparion* first occurs, that is of the order of approximately 15 Myr (ref. 8). These specimens belong to a distinctive group of *Hipparion*, also represented in the Old World, that have a well-developed and anteriorly-rimmed preorbital fossa. A detailed discussion of the taxonomic significance of preorbital fossae in *Hipparion* is the subject of another report[9]. The dentition is brachyodont relative to other *Hipparion*. The upper molars are characterised by the distinctive *Hipparion* isolated protocone, which in the sample from Trinity River Pit 1 is rounded in cross section. The plications on the fossette borders are simple relative to other *Hipparion*. The lower cheek teeth have separated metaconids and metastylids and either deep externomedial valleys (ectoflexids) or weak pli-caballinids.

It is generally accepted that *Hipparion* arose in the New World during the Miocene from some species of *Merychippus* and subsequently dispersed to the Old World[10,11]. The *Hipparion* from the early Barstovian of Texas represents the earliest-known member of this group. It is primitive in several aspects, including small size, rounded protocone, and brachyodonty, and advanced in the development of the diagnostic anteriorly-rimmed preorbital fossa, which is also found in later representatives of this group[9]. In younger Clarendonian sediments of the New World,

Fig. 1 Earliest known *Hipparion*, from Trinity River Pit 1, Fleming Formation, early Barstovian, San Jacinto County, Texas Gulf Coastal Plain. *a*, F:AM 73940, skull that shows preorbital fossa. *b*, F:AM 73940, left upper cheek teeth. *c*, F:AM 73940, left lower cheek teeth. F:AM is the abbreviation for; Frick: American Mammals, The American Museum of Natural History.



Hipparion is represented by a few morphologically distinct groups that can be characterised by differences in the development of preorbital fossae, and, to a lesser extent, differences in dental patterns. In the Neogene of the Old World, *Hipparion* first appears at the base of the Vallesian, which represents in part the same time interval as the Clarendonian of the New World[2]. The presence of *Hipparion* in the Old World seems to represent dispersal from the New World. Based on characters of the dentition, it has been argued that *Hipparion* in the Vallesian of the Old World was monophyletically represented by one species, *H. primigenium*. It has also been hypothesised that *Hipparion* underwent an adaptive radiation during Vallesian and later time that resulted in the multitude of species described in the literature[12]. But, based on differences in preorbital fossae in Holarctic *Hipparion* we suggest that clearly differentiated forms of *Hipparion* were already in existence in the New World by Clarendonian time, and dispersal to the Old World could have been polyphyletic with the presence of several forms during Vallesian and later time.

Some early workers used the first appearance of *Hipparion* in Holarctica as the herald of the Pliocene[13,14]. With the advent of more recent biostratigraphic and geochronologic interpretations, this hypothesis is no longer considered valid by most workers[1,2]. Although the *Hipparion* datum does not correspond to the Mio-Pliocene boundary, it is still used as a geologically synchronous event for purposes of correlation in the western Old World. The presence of *Hipparion* in early Barstovian sediments of the New World is significantly older than the first appearance of *Hipparion* in the Vallesian of the western Old World. The question now arises, to what palaeogeographic extent should the *Hipparion* datum be restricted in order to retain its usefulness as a geologically synchronous event? Much evidence has been presented to demonstrate the usefulness of the *Hipparion* datum in the western Old World and Africa. Precise geochronological studies are needed to determine when and what form(s) of *Hipparion* first appeared in the eastern Old World. If the first appearance of *Hipparion* is not a geologically synchronous event throughout the Old World, or if it represents dispersal of several forms, then it would not be necessary to reject the *Hipparion* datum concept, but it would have to be restricted to some palaeogeographically finite area.

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Tail autotomy, functional conflicts and their resolution by a salamander

IF an organ serves several functions, two or more of these may conflict at times in their contribution to the immediate fitness of the organism. Among lower invertebrates the tail is commonly used as a defence against predators but it has other functions such as fat storage, locomotion, respiration and courtship. Experiments have shown that tail loss can depress reproductive output in lizards[1,2], but this has not been reported for natural populations. Data reported here suggest that loss of the tail frequently depresses reproductive output in natural populations of the plethodontid salamander *Batrachoseps attenuatus*, which is widespread in California. The dual use of the tail for energy storage and for protection may increase the adaptive flexibility of the salamander for coping with an unpredictable environment.

B. attenuatus has a tail which may reach twice the trunk length. It commonly represents 50% of the biomass of mature animals, and can provide proteins and lipids for maintenance, reproduction and continued growth in trunk length. During the dry summer months *B. attenuatus* retreats underground and does little or no feeding[3]. At this time females produce eggs and males come into full breeding conditions (large premaxillary teeth develop and neck muscles enlarge). During the dry season, the mean tail volume of mature individuals has decreased by 29%. Thus the tail is presumably an important energy source for maintenance and reproduction during this period.

Tail autotomy seems to be an adaptation for escaping from predators. It has been associated with attacks from snake predators in the laboratory (my unpublished results and personal communications from S. Arnold). Although autotomy may be associated with intraspecific competition, I have seen no evidence for this during experiments on competition for burrow sites. The tail of *B. attenuatus* can drop off at any segment; it is then regenerated at apparently a constant rate until it approaches its full length[4]. Estimates of annual tail breakage in populations in or near Berkeley, California, range from 28 to 48% (data to be published elsewhere). For only half the time, however, does a salamander lose more than 30% of its tail. Size and sex classes do not differ significantly in the frequency of observed tail breaks.

Three lines of evidence suggest that tail autotomy inhibits reproduction in natural populations of this salamander. First, mature individuals, both males and females, which fail to breed during one season have significantly shorter tails than do breeding individuals of similar trunk length (Table 1). Both males and females typically reproduce annually in the San Francisco Bay area; the percentage not breeding ranged from 0 to 18% per year for the populations included in my study. Since most of these non-breeding individuals (65% compared with 17% of breeding individuals) had incompletely regenerated tails (estimated as less than 75% the length of unbroken or fully regenerated tails), autotomy, subsequent regeneration or both are interpreted as major depressants of reproductive output in these populations.

Second, maturity may be delayed as a consequence of tail autotomy. In all but one comparison, immature animals, similar to breeding individuals in trunk length and presumably age, had significantly shorter tails (Table 1) due to incomplete regeneration (25% compared with 3% of breeding individuals).

Third, field evidence suggests that tail autotomy not only inhibits reproduction but also reduces the amount of reproductive output for individuals which do reproduce.

Table 1 Comparison of relative tail length between breeding and nonbreeding salamanders of similar trunk length

	Redwood forest		Conifer woodland		Eucalyptus woodland	
	Females	Males	Females	Males	Females	Males
Not breeding, immature	− 1.8 (23)	− 5.7 (26)	− 7.7 (20)	− 1.4 (11)	—	—
Breeding	1.8 (12)	− 0.5 (14)	− 0.6 (22)	1.2 (19)	—	—
Probability	0.12	0.03	0.0015	0.07		
Not breeding, mature	− 9.2 (13)	−12.8 (21)	−23.2 (6)	−18.3 (1)	−26.1 (1)	−23.8 (4)
Breeding	− 2.1 (22)	− 4.4 (25)	− 5.2 (14)	−16.5 (3)	− 5.2 (4)	− 8.8 (4)
Probability	0.02	0.0004	0.0003	0.94	0.0001	0.135

Values are the means of the deviations (mm) of observed tail lengths from the expected unbroken tail length, calculated from the linear regression of tail length on trunk length for individuals that had unbroken or fully regenerated tails. These regressions were based on samples collected in the same habitats at different times than the samples used in this analysis. Only breeding individuals that were within 1 mm of the trunk length of nonbreeding individuals in the same collection were used. Data for each habitat are pooled from samples collected over several years. Numbers in parentheses are sample sizes. Student's *t* test was used to determine the significance of the differences; probabilities are for a two-tailed test.

In all but one case, tail length showed a significant positive partial correlation with an index of reproductive effort (the number of ovarian eggs of gravid females and the extent to which the neck muscles are enlarged in males, expressed as the ratio of neck width to head width) when trunk length is constant (Table 2).

The inhibitory influence of tail loss on reproductive output may derive simply from loss of considerable energy reserves available in the long tail or from use of remaining energy reserves, such as those in abdominal fat bodies or in the tail remnant, to regenerate the tail during the dry season instead of reproducing. Hendrickson[4] concluded on the basis of recaptures of marked animals that regeneration occurs during both the wet and dry seasons. To test the possibility that tail regeneration during the dry season depresses reproductive output I collected 57 salamanders before the dry season (early May) and removed the whole tail from 27 of them; the rest served as controls. The salamanders were maintained, unfed, on a natural photoperiod at 13 °C until September, at which time they were killed and examined for tail regeneration and reproductive condition. (Forty salamanders, which had been collected on a different day than the others, died shortly after the start of the experiment, probably from heat stress when collected[5].)

Table 2 Partial correlation coefficients of trunk length and tail length with an index of reproductive effort

	Redwood forest	Conifer woodland	Eucalyptus woodland
Females			
Sample size	42	78	46
$r_{\mathrm{SVL,RI.TL}}$	0.53 ($P < 0.001$)	0.64 ($P < 0.001$)	0.77 ($P < 0.001$)
$r_{\mathrm{TL,RI.SVL}}$	0.36 ($P < 0.05$)	0.34 ($P < 0.01$)	0.55 ($P < 0.001$)
Males			
Sample size	28	34	
$r_{\mathrm{SVL,RI.TL}}$	0.40 ($P < 0.05$)	0.53 ($P < 0.01$)	
$r_{\mathrm{TL,RI.SVL}}$	0.22 ($P = 0.32$)	0.55 ($P < 0.01$)	

Both tail length and the index of reproductive effort are a linear function of trunk length. Calculations include only actively breeding individuals. SVL, trunk length; TL, tail length; RI, reproductive index (see text for explanation).

Clearly the salamanders regenerate the tail at some expense of reproduction. All experimental animals ($n=9$) regenerated their tails at an average rate of 2.6 mm per month. All but one of them failed to become fully reproductive; all controls ($n=6$) became reproductive ($P=0.001$, Fisher's exact test).

Thus, field observations and one experiment suggest that autotomy of the tail depresses reproductive output, at least in part, because the regeneration is taking place when energy reserves are needed for reproduction. I conclude that the use of the tail for defence has functional priority over its use to supply energy for reproduction. An explanation for this priority is that the environment has selected for longevity in *B. attenuatus* at the expense of reproduction when the two selective pressures are in conflict[6]. The environment is unpredictable to this salamander with respect to both the length of the dry season and the amount of rain during the wet season. Variability in rainfall produces variability in the amount of energy an adult can store for reproducton during the dry season. After a wet season with half the normal rainfall (1971–72), females in two habitats had 6% and 11% fewer eggs ($P=0.12$ and 0.0002, respectively, one-tailed *t* test) than after a wetter than normal season (1972–73). Variability in the length of the dry season is thought to produce variability in mortality, particularly in juvenile mortality[6]. Averaging ten times the weight of first year juveniles, adults have a metabolic advantage in surviving prolonged periods without food, and thus survival is more certain for them than for juveniles. Both variability in the production of young and uncertainty in their survival are predicted to favour increased longevity at the expense of reproduction[7].

It is not surprising that tail regeneration (and therefore defence) has priority over reproduction, for such priority is theoretically expected when selection favours longevity. It is more perplexing that the salamander does not use some other structure for either energy storage or predator defence. Although separate systems may have been 'evolutionarily available' to species, the dual use of the tail may be adaptive in that it can increase the responses of the salamander in allocating energy for reproductive and somatic functions. A specialised defence system, such as a toxin, costs the salamander a certain amount of energy that is then irrevocably tied up in the system. In contrast, by using a structure for defence that can also be used to accumulate required energy reserves, the salamander can draw on these reserves for reproduction if they are not

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1230/20

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used in defence. When energy availability fluctuates greatly, it seems adaptive to have an occasional opportunity to expend a maximal amount of energy for reproduction.

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Coral-snake pattern recognition and stimulus generalisation by naive great kiskadees (Aves: Tyrannidae)

THE neotropical coral-snake complex with its contrasting ringed patterns includes true coral snakes, for example, the highly venomous elapid genus *Micrurus*, and many colubrids that are either less strongly venomous or non-venomous[1]. Whether or not this complex involves Batesian and/or Müllerian mimicry has been widely debated[2–8]. Wickler[1] suggested that if mimicry depends on predator learning, true coral snakes are too deadly to be models; rather, they are mimics of the less dangerous colubrids of the complex (Mertensian mimicry). He also claimed that there was no evidence that any predator could recognise a coral snake innately[1]. I have shown that Costa Rican turquoise-browed motmots (*Emomota superciliosa*) need no learning to show strong aversion to a pattern of wide yellow and narrow red rings[6]; this I have interpreted as an innate recognition of a generalised coral-snake pattern. No Costa Rican member of the coral-snake complex has wider yellow than red rings, however, so I had no proof that the motmots were not simply showing an aversion to a general aposematic pattern. I present here evidence that another avian predator needs no learning to avoid not only a pattern of wide yellow and red rings, but also the most common local *Micrurus* pattern of red, yellow and black rings.

The great kiskadee (*Pitangus sulphuratus*) occurs in open and semi-open habitats from southern United States (Texas) to south Argentina. It regularly eats small reptiles[5], and *Micrurus* and many other members of the complex occur over much of its range. These snakes could be dangerous to kiskadees. *Micrurus* in particular may lie partly concealed in leaf litter during the day, often with its head under leaves. A kiskadee attacking the exposed portion of such a snake would be in danger of being bitten, especially if that portion did not include the head. As kiskadees lack heavy protecting scutes on their legs, such a bite could well prove fatal.

Six young kiskadees from three broods aged 12–20 d were removed in May 1976 from their nests in north-western Costa Rica and raised in the laboratory in 91 × 46 × 46-cm hardware cloth cages. Two cages each held two birds; the other two held one each. Experiments in these cages were begun when the kiskadees were old enough to catch their own prey, that is, 45 d. In each experiment a wooden dowel model 6 cm long and 1 cm in diameter was placed on the cage floor and the number and location of the birds' pecks was recorded for 5 min. Models were painted with non-toxic tempera colours in the following five patterns: white with narrow green rings; yellow with narrow red rings; yellow with narrow red stripes; 'coral-snake' rings (wide red, narrow yellow, wide black, narrow yellow); and coral-snake stripes. Two models were made for each pattern: a 'solid' model completely covered with the pattern, and an 'end-third' model having only one end painted, the other two thirds being plain wood (Fig. 1). With the exception mentioned below, all birds received the patterns in the above order.

Each kiskadee had previously attacked solid models of plain white, green, red, yellow and black without hesitation. Of the five patterns on solid models, two were completely avoided, two readily attacked, and one attacked with great caution (Table 1). Although no kiskadee approached either the yellow and red ring or the coral-snake ring model, the responses to the two differed. Only two birds gave mild alarm calls to the former; the same two plus at least two more gave high intensity alarm calls (like those given by a captured bird) to the latter. Their response to the white and green ring model (the first pattern any bird received) shows that they did not fear ring patterns in general. Nor did they hesitate to attack the yellow and red stripe model, which has a relative proportion of yellow and red identical to that of the ring pattern. Unexpectedly, although all six birds approached the solid coral-snake stripe model, all showed great hesitation, and the four birds that finally did attack did so by darting forward with spread wings and contour feathers tightly in.

With the end-third models, the birds directed most of their pecks to the painted third of the white and green ring, the yellow and red stripe, and the coral-snake stripe models, but gave a significant response to the end farthest away from both the yellow and red ring and the coral-snake ring models (Table 1). Kiskadees thus consistently avoid the coral-snake ring and the yellow and red ring patterns, but hesitate to attack the coral-snake stripe pattern only when it covers the entire model.

This aversion to the two ring patterns seems to require no learning. Kiskadees have covered nests, and the three youngest birds barely had their eyes open when they were taken, so it is unlikely that they perceived any patterns in the dim light within their nest before their capture.

Coppinger[9] found that naive passerines of three species gave alarm calls and active escape behaviour to a large variety of novel stimuli, the degree of rejection depending on the amount of stimulus change and the birds' previous experience. The order in which the kiskadees received the

Fig. 1 Models used in the experiments. *a*, Solid forms. *b*, End-third forms. The basic patterns were: 1, white and green (or yellow and red) rings; 2, yellow and red stripes; 3, coral snake rings, and 4, coral snake stripes. The three sections of the end-third models were recorded as p, m and u.

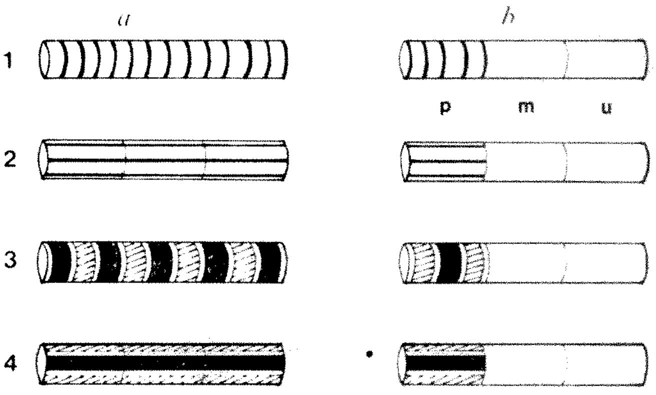

Table 1 Kiskadees' pecks to the ten models

Pattern	Pecks at solid models	Pecks at end-third models				
		% at each area				
		p	m	u	Total pecks	χ^{2}*
White and green ring	59	100	—	—	51	p
Yellow and red ring	—	17.86	—	82.14	28	u
Yellow and red stripe	44	100	—	—	45	p
Coral-snake ring	—	11.11	—	88.89	9	u
Coral-snake stripe	7	100	—	—	23	p

*p indicates a response to the painted third at $P<0.01$; u indicates a response to the unpainted end at $P<0.05$.

models was designed to test for such novelty effects. The first pattern given was the white and green ring, which all birds attacked without hesitation: neither fear of patterns themselves nor fear of rings can thus be attributed to novelty. Because the three oldest birds had received the coral-snake ring model before the coral-snake stripe model, however, their hesitation to attack the latter might have been due to their earlier experience. The three youngest birds were therefore given the stripes before the rings. They responded in a manner similar to that of the older birds (Table 1), showing that this hesitation was not caused by previous experience.

The kiskadees' avoidance of the two ring patterns thus seems to be innate. It is unlikely that two separate innate aversions are involved; their stronger rejection of the coral-snake models, both rings and stripes, suggests that great kiskadees have an innate aversion to the coral-snake pattern, yet will generalise to avoid a pattern of wide yellow and narrow red rings.

Pattern generalisation by predators is essential for the functioning and evolution of both Batesian and Mullerian mimicry[1,10]. If mimics evolve by microevolution, broad stimulus generalisation by local predators is necessary to give selective advantage to early divergent phenotypes just beginning to approach the model in pattern. For predators that have learned to avoid a pattern, such generalisation is well documented[11-12]. My results show that generalisation can also occur with an innate aversion. In the presence of such predators, classical Batesion or Müllerian mimics can theoretically evolve even when the model is lethal; furthermore, for Batesian mimics, the model need not be numerically more abundant than the mimic[13]. If innate recognition and generalisation of the coral snake pattern is a common phenomenon, the Mertensian mimicry hypothesis is unnecessary to explain the evolution and maintenance of the coral snake mimicry complex.

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Divergent taste responsiveness to fruit of the tree *Antidesma bunius*

GENETIC divergence of taste responsiveness was first demonstrated for phenylthiocarbamide (PTC)[1], the inability to taste PTC bitter being described as an inherited Mendelian recessive character, and ability to taste PTC bitter as dominant[2]. This hypothesis has largely been confirmed[3,4] although occasional incomplete penetrance of the 'taster' gene has been reported. Recently, eight people were served with a pie made from antidesma berries, and two people complained that the pie was extremely bitter and inedible. The other six people, however, found the pie pleasant tasting, enjoyably edible and sweet. This incident prompted us to survey taste responsivness to antidesma. We found that all subjects who tasted antidesma as bitter found PTC not bitter, whereas no subject who tasted PTC as bitter found antidesma bitter.

Fresh berries from the tree *Antidesma bunius* were pressed and an aqueous extract derived. The extract was stored at 4 °C. A saturated solution of PTC (Baker) was also prepared. For the test each solution was placed into a 60-ml dark-coloured glass-dropper bottle fitted with a glass dropper.

The subjects were 170 volunteers (88 males) from the greater Washington DC area, aged 7–89 yr. The group included eight blacks, two orientals and 160 Caucasians of diverse national origin. To test taste responsiveness one drop of antidesma extract and PTC was placed successively on the tongue and the subject was requested to taste it. Each subject was also asked whether the drop tasted salty, bitter, sweet or sour and to judge the intensity of its saltiness, sweetness, sourness or bitterness on a scale from 1–100 based on previous taste experience[5]. The antidesma extract was always given first and was followed by the PTC within 5 min. Age and sex of each subject were noted as was the presence of ichthyosis. A modified form of the Ishihara colour plates[6] was shown to each subject in a standard manner in order to identify the presence of colour blindness.

Responders to antidesma extract and to PTC were defined as subjects who described the respective solutions as bitter; non-responders were defined as those who described it either as tasteless or another taste quality. Intensity responses were calculated as the median and the mean responses of the taste responders or non-responders for each respective group.

Responders to antidesma extract comprised approximately 15% of the subjects tested, non-responders, 85% (Table 1); responders to PTC comprised approximately 68% of the group tested, non-responders, 32% (Table 1). There was no relationship in responsiveness to either antidesma or PTC with age, sex, race, national origin, colour blindness or ichthyosis. Most antidesma responders judged antidesma as bitter, the mean response being about 39% as bitter as the bitterest substance ever tasted. Most PTC responders judged PTC as about twice as bitter as the antidesma responders judged antidesma, the mean responsiveness being approximately 82%. Among the 145 non-responders to antidesma, 67 stated that it was slightly sour, 39% that it was sweet,

Table 1 Comparison of PTC and antidesma responders and non-responders

	Responders	Non-responders
PTC		
Subjects	115	55
Sex	57 males; 69 females	31 males; 22 females
Mean age (yr, range)	44 (9–78)	41 (7–89)
Mean response intensity (%, range)	81.6 (5–100)	1.5 (0–30)
Median response intensity (%)	100	0
Median response quality (response, no.)	Bitter (115)	Tasteless (47)
Mean response frequency (%)	67.6	32.4
Antidesma		
Subjects	25	145
Sex	16 males; 9 females	71 males; 83 females
Mean age (yr, range)	43 (7–89)	42 (9–78)
Mean response intensity (%, range)	39 (5–100)	24 (0–100)
Median response intensity (%)	20	12
Median response quality (response, no.)	Bitter (25)	Sour (67)
Mean response frequency (%)	14.7	85.3

29 stated that it was salty and 10 could not designate any specific taste quality.

Among the 25 responders to antidesma there were no responders to PTC and, conversely, among the 115 responders to PTC there were no antidesma responders.

Three families in which antidesma responsiveness was identified were studied. In each family the father was the index subject identified. In one family, the wife and each of the six children (three girls, three boys) were antidesma non-responders. In another family, the wife and two children (one girl, one boy) were antidesma non-responders. In the last family, the wife, paternal mother and father and three children (one girl, two boys) were studied; the paternal father and one son, an addition to the father previously identified, were antidesma responders. Among the latter two families there were no genetic markers to characterise the antidesma responders with respect to red blood cell group, type, phenotype, Rh factor or plasma Xga. One family in which both husband and wife were PTC non-responders was studied; both were antidesma non-responders and both of their children (one boy, one girl) were PTC and antidesma non-responders.

Although taste divergence for antidesma extract has not been described before, the percentage of subjects in whom taste responsiveness to PTC was found in this survey agrees closely with those of previous investigators[7,8] as do the response characteristics of the subjects studied[9]. The active substance responsible for the taste responsiveness of the antidesma extract has not been identified although it is water soluble and heat stable. The genetics by which taste divergence to antidesma is determined is unclear, although it seems to occur among PTC non-responders comprising about half of this group. This suggests that the bitter response to antidesma among PTC non-responders bears some relationship to those factors which determine PTC taste responsiveness either on a functional or genetic level.

The divergence of taste responsiveness to antidesma raises several questions. First, antidesma fruit, unlike PTC, has been used as food by natives in South-East Asia and Florida for many years and has been eaten in pies, jams and sauces or as raw fruit. Divergent tastes to this food raises the question of the presence of other substances which may appear in the diet in which individual differences in taste perception and hence, preference, may occur. Although bitter tasting substances are not usually preferred as food or drink, preferences of the majority may exert significant influence on food practises. Thus, the number of substances to which divergent taste responses may occur and how much they may influence food and drink preference and intake are unknown. Second, these data raise the question of whether preferences for some food and drink may be determined, in part, by genetic factors. Third, individual taste responsiveness to various naturally occurring substances may serve as useful markers to individual differences, not only to food and drink preferences, but also to drug responsiveness[12] and sex differences[13]. Lastly, divergent taste responsiveness to antidesma and PTC as contrasted with the more usual uniform bitter taste responsiveness to most alkaloids[14], may be useful in the study of the psychophysical and biochemical characteristics of bitterness.

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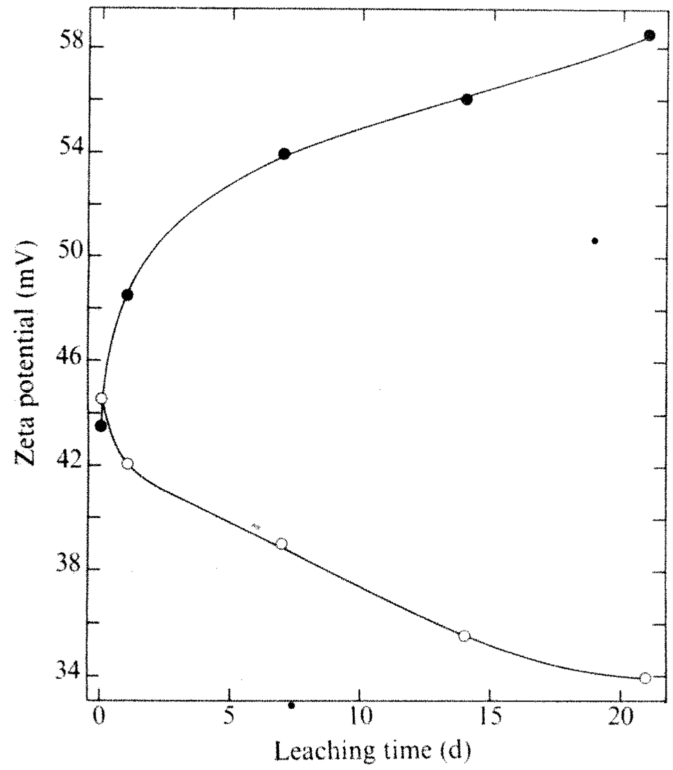
Surface charge and asbestos toxicity

Asbestos is a serious occupational and environmental hazard because of its fibrogenic and carcinogenic actions on the lung. Asbestos fibres exist in various physical forms, all of which are toxic. It is not known, however, which form is the most hazardous or which fibre property is the most important for inducing the toxic effects. It is thus important to examine relationships between the structure of the fibres and their biological activity. We have investigated the relationship between the surface charge and the haemolytic activity (ability to rupture the erythrocyte membrane) of asbestos. We report here that different forms of asbestos can be activated or inactivated by surface charge alterations.

Asbestos fibres are divided into two classes, chrysotiles and amphiboles, on the basis of their crystal structure[1,2]. Chrysotiles are characterised by hollow cylindrical fibres formed by spiralling a double sheet of silica and brucite ($Mg(OH)_2$) with the brucite on the outer surface. Amphiboles are characterised by fibres formed by stacking double silica chains back-to-back with a layer of hydrated cations in between. Owing to their dissimilar crystal structure and chemical composition, the two classes also differ in their physicochemical properties. These differences are reflected in short-term *in vitro* effects, chrysotiles being more cytotoxic to macrophages[1,3,4] and more haemolytic[1,5–7] than amphiboles. For long-term *in vivo* toxicity, however, there is no clear specificity in the fibrogenic and the carcinogenic activities of the two classes[1,8–11]. This paradox of short-term and long-term asbestos toxicity has not been resolved, but factors such as surface charge[12–17] and dimensions[11,18,19] of the fibres, deposition and retention of the fibres in the lung[19,20], coating[21] and dissolution[22] of the fibres by lung secretions, and susceptibility of the fibres to phagocytosis[1,8] may be significant. We have suggested that the haemolytic activity of asbestos is principally determined by the absolute magnitude of the surface charge that the fibres acquire in solution[23]. For chrysotiles the surface charge is associated with structural magnesium atoms which are known to be leached from the fibre surface *in vivo*[22], suggesting that progressive shifts in absolute surface charge could account for the discrepancy in short-term and long-term asbestos toxicity. We report here the effect of leaching on the surface charge and the haemolytic activity of representative samples of both classes of asbestos.

Surface charge, represented by the zeta potential[24], and haemolytic activity were measured for two asbestos reference samples of the International Union against Cancer (UICC) (ref. 25)—chrysotile A and the amphibole crocidolite. The fibres were leached as 0.5% (w/v) suspensions in Tyrode's (physiological buffer) solution, 0.01 M HCl or 0.1 M HCl. On each day of a leaching period, the fibres were centrifuged (3,090*g*, 20 min) and the leachate was siphoned and replaced. The HCl leachings were for 1 d. The electrophoretic mobilities of fibres incubated for 2 h as 0.01% (w/v) distilled-water suspensions were measured using a microelectrophoresis instrument (Zeta-Meter). From these data, zeta potentials were approximated using the Helmholtz-Smoluchowski equation[24] which is generally considered to apply for cylindrical particles placed axially to the electric field. For the haemolysis tests, fibre samples of equal surface area (20 mg crocidolite, 7.7 mg chrysotile A) were incubated for 1 h in 2 ml of Tyrode's solution; 2 ml of a 1.5% (v/v) Tyrode's-solution suspension (*p*H 7.4) of washed sheep erythrocytes was then added. The mixtures were inverted twice every 10 min for 1 h at 23 °C. The optical densities were measured at 541 nm after centrifugation (495*g*, 20 min).

Fig. 1 Zeta potential in absolute units (*Z*) at *p*H 7.4 for asbestos fibres leached with Tyrode's solution. ○, UICC chrysotile A; ●, UICC crocidolite.



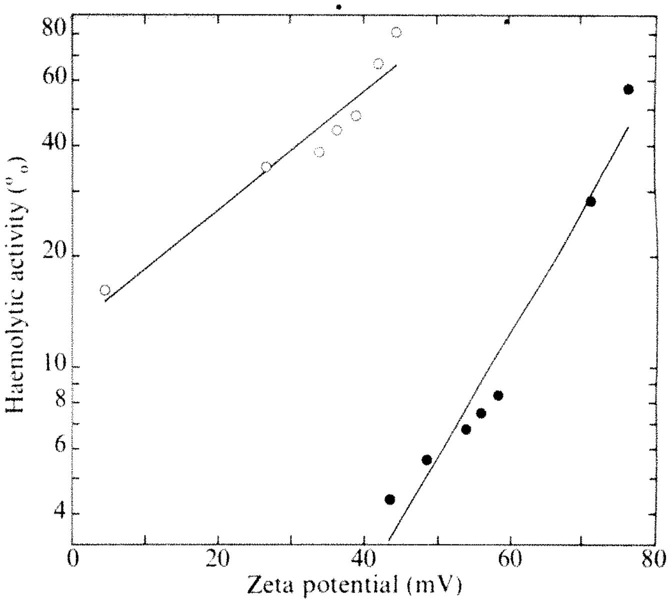


Fig. 2 Correlation between zeta potential in absolute units (*Z*) and haemolytic activity for leached asbestos fibres. ○, UICC chrysotile A (*r* = 0.96); ●, UICC crocidolite (*r* = 0.97).

Zeta potentials at *p*H 7.4 for the two types of unleached asbestos were similar in absolute magnitude, but opposite in polarity: +44.5 mV for chrysotile A and −43.5 mV for crocidolite. On leaching, the absolute magnitude of the zeta potential (*Z*) decreased for chrysotile A, and increased for crocidolite. The rate of change in *Z* at *p*H 7.4 for chrysotile A and crocidolite leached with Tyrode's solution is shown in Fig. 1. After a sharp change in *Z* during the first 5 d, the variation became nearly linear and at 21 d the *Z* values for chrysotile A and crocidolite were 34.0 and 58.5 mV, respectively. For both chrysotile A and crocidolite, there was a proportional shift in haemolytic activity as *Z* changed on leaching. Data for fibres leached with HCl and Tyrode's solution (Fig. 2) show the highly significant ($P<0.01$) correlation between *Z* and haemolytic activity for the two types of asbestos.

These results confirm the importance of surface charge to haemolytic activity and show that fibre dissolution can markedly alter the magnitude of surface charge and hence, activate or inactivate the fibres. The positively charged chrysotile was initially more active than the negatively charged amphibole. Leaching of the fibres caused the absolute magnitude of surface charge and the corresponding haemolytic activity of the chrysotile to decrease progressively and that of the amphibole to increase progressively. With continuous leaching *in vivo*, surface charge could be altered so that the biological activities of chrysotiles and amphiboles converged. This phenomenon could explain why the two classes of asbestos fibres, although different in their short-term *in vitro* effects, are not clearly different in their long-term toxicities.

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Suppressor cells in experimental trypanosomiasis

HUMAN and experimental forms of African trypanosomiasis are characterised by a profound suppression of immunological responsiveness. Suppression of both humoral and cell-mediated responses has been observed. *Trypanosoma gambiense* infections in man reduce antibody responses to *Salmonella typhi* (H antigen) and skin reactivity to purified protein derivative, *Candida* and dinitrochlorobenzene[1]. *T. brucei* infections in mice cause marked suppression of antibody[2] and splenic plaque-forming cell[3,4] responses to sheep red blood cells (SRBC), and in rabbits suppress the development of experimental allergic neuritis[5]. The mechanism underlying this inhibition of immunocompetent cell function has not been elucidated. We have examined the DNA synthetic responses of spleen cells from *T. brucei*-infected mice to concanavalin A (con A) and phytohaemagglutinin (PHA) (both T-cell mitogens), *Escherichia coli* lipopolysaccharide (LPS, a B-cell mitogen) and allogeneic cells, and the ability of these cells to influence the responses of normal cells. We present evidence suggesting that suppressor cells[6,7] are involved in the immunological hyporesponsiveness observed in this disease.

Infection of CBA/CaJ mice with S 42 strain *T. brucei* produced a progressive and uniformly fatal infection (Fig. 1). After the inoculation of 10^4 trypanosomes (all infections were initiated by the intraperitoneal inoculation of 10^4 trypanosomes derived from stabilate[8] material) parasites were detected in the peripheral blood by day 3 and increased rapidly until day 6. Parasite levels then regressed until day 8 or 9, after which they increased steadily and proved fatal in 30–35 d.

DNA synthetic responses of spleen cells from infected mice to con A, PHA and LPS were suppressed as early as day 3 of infection (Fig. 1) and the suppression increased as the infection progressed. The degree of suppression

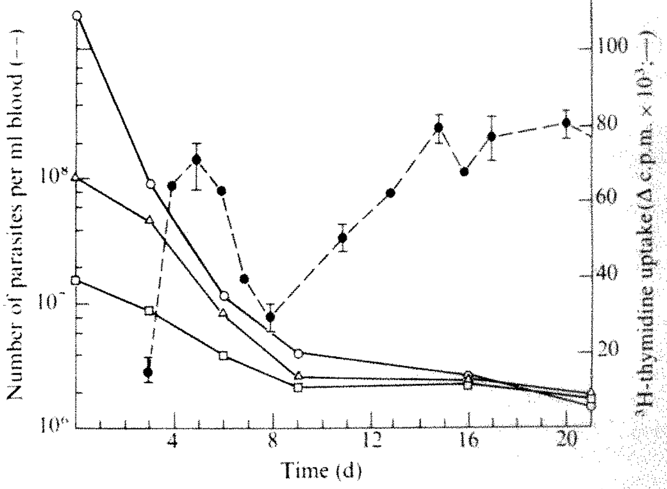


Fig. 1 Mean parasitaemia (●) ± 1s.e. and mean DNA synthetic responses to con A (○), PHA (□) and LPS (△) of spleen cells of CBA/CaJ mice infected with S 42 *T. brucei*. Standard errors were < 5% in all cases. Mean parasitaemia was estimated from haemocytometer counts made on the peripheral blood of six mice. DNA synthesis was measured by Williams[9] method. Viable spleen cells (5×10^5) were cultured in 0.1 ml RPMI 1640 containing 5% foetal calf serum (Gibco) with optimal concentrations of mitogens. Con A (Calbiochem) was used at a final concentration of 2 µg ml^{-1}, PHA (Difco) at 0.31 µl ml^{-1} and LPS (LPS 055:B5, Difco) at 50 µg ml^{-1}. All cultures were set up in flat-bottomed tissue culture plates (Linbro) and were incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cultures were pulsed with 1 µCi ^{3}H-thymidine (New England Nuclear, specific activity 5 Ci mmol^{-1}) 16 h before collection. At each stage of infection, spleen cells were pooled from groups of three mice and cultures were set up in triplicate. Results are expressed as mean c.p.m. in stimulated cultures minus mean c.p.m. in unstimulated cultures (Δ c.p.m.).

observed did not always seem to be directly proportional to blood parasitaemia. The ability of spleen cells from infected CBA/CaJ mice to respond to allogeneic (BALB/c) spleen cells in a mixed lymphocyte reaction was also significantly inhibited (data not shown).

As recent work[10,11] has implicated suppressor cells with adherent properties in a comparable situation—the reduced immunological reactivity obtained in animals treated systemically with large doses of protein antigen—we examined the possibility that the nonspecific suppression in *T. brucei*-infected mice was mediated by an analogous mechanism.

Initially, we examined the effect of adding various number (6×10^4, 1.25×10^5, 2.5×10^5, 5×10^5 and 7.5×10^5) of spleen cells from infected mice (at day 12 of infection) or normal mice to cultures of normal spleen cells (5×10^5) stimulated with con A, PHA, or LPS. Spleen cells from infected mice caused consistent, dose-dependent, suppression of the mitogen-induced DNA synthetic responses of the normal cells. Addition of normal spleen cells caused no significant suppression except at the highest cell concentration and this was probably due to 'crowding' effects. Spleen cells from infected mice also caused significant suppression of one-way mixed lymphocyte reactions between normal CBA/CaJ and mitomycin C-treated BALB/c spleen cells. The degree to which the DNA synthetic response of normal cells to con A, PHA, LPS and allogeneic cells was suppressed at a $1(2.5 \times 10^5)$: $2(5 \times 10^5)$ ratio of infected to normal cells is shown in Fig. 2. The ability of 2.5×10^5 spleen cells from infected CBA/CaJ mice to suppress the responses of 5×10^5 normal spleen cells increased as the infection progressed. We found that the con A-induced DNA synthetic response was reduced to 50% of the normal response by day 6 cells, to 19% by day 12 cells and to 11% by day 20 cells.

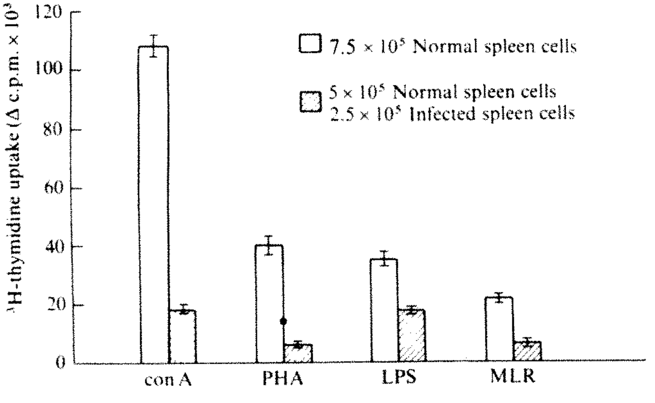
We next examined the effect of depleting *T. brucei* spleen cells of glass-wool-adherent cells. DNA synthetic responses of whole and nonadherent spleen cells from normal and infected mice to con A, PHA and LPS are shown in Fig. 3. Depletion of glass-wool-adherent cells caused a more substantial increase in the response of *T.brucei* spleen cells than of normal cells to PHA and LPS; the response to con A was improved, but to a lesser extent by this procedure. We have also found that glass-wool-adherent cells from infected mice consistently suppressed mitogen-induced responses of normal spleen cells 25–30% more than the unfractionated population; peritoneal macrophages from infected mice had no effect. Hence the suppressor cell seemed to have adherent properties but was distinct from the peritoneal macrophage.

It seemed possible, however, that some of the suppression observed was a direct inhibitory effect of trypanosomes present in spleen cell suspensions from infected mice—at day 12 of infection, the spleen cell to trypanosome ratio was approximately 30 : 1. To investigate this possibility we separated trypanosomes from the peripheral blood of infected mice[13] and added trypanosomes (4×10^3–2×10^5) to cultures of normal spleen cells (5×10^5) which were then stimulated with con A or LPS: these numbers of trypanosomes caused no suppression, indicating that the suppression in our cell mixture experiments was mediated primarily by a cell. This was substantiated by the observation that glass-wool-adherent cells, although more suppressive, were contaminated by 1/10 the number of trypanosomes present in the whole spleen cell population. In more recent experiments we have observed that very large numbers of trypanosomes (6×10^5 or more) suppress mitogen-induced responses of normal cells. The possibility that this suppression is also due to the induction of suppressor cell activity cannot be excluded.

Although the cell mixture and glass wool fractionation experiments clearly implicate a splenic cell in the suppression, it is possible that the presence of some trypanosome antigen in culture may augment the suppression observed. Gershon, Gery and Waksman[10] found that the reduced responsiveness to PHA *in vitro* of spleen cells from animals immunised with SRBC, bovine γ-globulin and bovine serum albumin was further suppressed by the addition of the immunising antigen to the cultures.

To characterise further the cell responsible for suppression in this system, we compared the growth of the parasite in normal and congenitally athymic (nude) BALB/c mice and tested the spleen cells of these mice for suppressor activity. Parasitaemias were generally lower in nude mice than in normal controls (Fig. 4*a*). Although spleen cells from infected BALB/c mice caused significant suppression of mitogen responses, spleen cells from infected nude mice at the same stage of infection (day 11) failed to suppress the mitogen-induced DNA synthetic responses of normal cell (Fig. 4*b*), indicating that the suppressor in the spleens of *T.brucei*-infected mice was either a T cell or was dependent on T-cell influence for its generation. Treatment of spleen cells from infected mice with an anti-Thy-1 antiserum and complement, however failed to remove their suppressive effect. There

Fig. 2 Effect of spleen cells from *T. brucei*-infected CBA/CaJ mice on con A,PHA,LPS and allogeneic-cell-induced DNA synthetic responses ± 1 s.e. of normal spleen cells. Mixed lymphocyte reactions (MLR) were initiated using 2.5×10^5 CBA/CaJ spleen cells and 2.5×10^5 mitomycin C-treated BALB/c spleen cells. Spleen cells (10^7 per ml) were treated with mitomycin C (30 μg ml^{-1}, Sigma) at 37 °C for 30 min. Culture conditions were as described previously. Cultures were pulsed with 1 μCi ^{3}H-thymidine 16 h before collection.



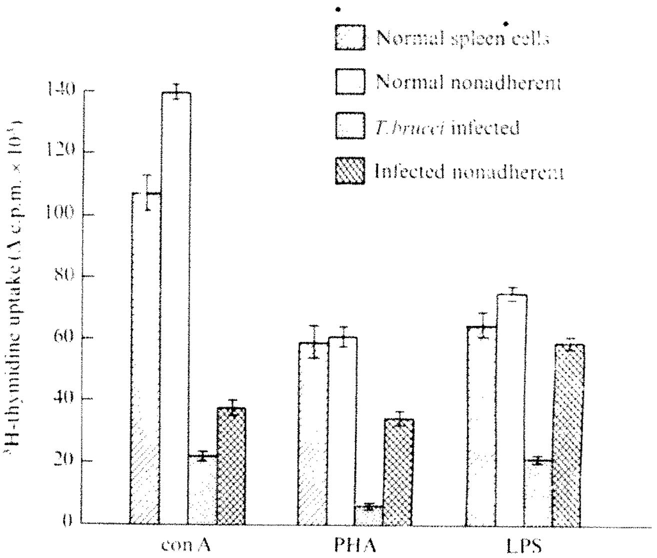
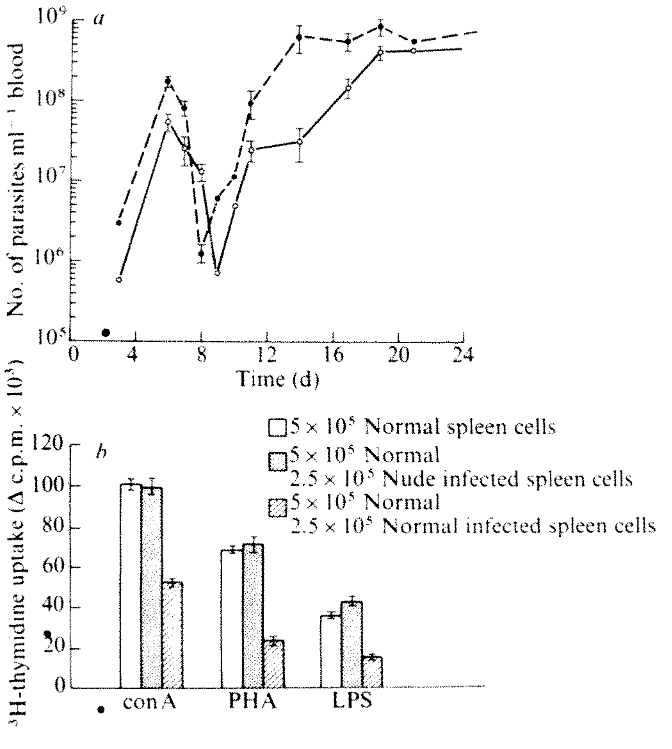


Fig. 3 DNA synthetic responses to con A,PHA and LPS (± 1 s.e.) of whole and nonadherent spleen cells from normal and *T. brucei*-infected CBA/CaJ mice. Spleen cells were pooled from four normal and four infected (day 7 of infection) mice. Adherent cells were removed on glass wool columns using the method of Folch, Yoshinaga and Waksman[12].

Fig. 4 Mean parasitaemias (± 1 s.e.) in normal (●) and nude (○) BALB/c mice infected with S 42 *T. brucei* (*a*) and the effect of spleen cells from these mice on con A, PHA and LPS-induced DNA synthetic responses of normal BALB/c spleen cells (*b*).



have been reports of suppressor T-cells which seem to express only small amounts of Thy-1 on their surface and are therefore difficult to lyse with anti-Thy-1 antisera[14]. Hence in spite of its insensitivity to the concentration of anti-Thy-1 antiserum which we used we cannot exclude the possibility that the suppressor in our system is a T cell.

We have also observed that unfractionated or T-cell-enriched (on nylon wool columns) spleen cells from infected mice cause marked suppression of the plaque-forming colony response of normal spleen cells to SRBC *in vitro* (unpublished results of D. D. Eardley and A.N.J.), and that the adoptive transfer of spleen cells from *T.brucei*-infected mice to normal recipients results in infections which are enhanced compared with infection in controls receiving normal spleen cells and the same number of parasites.

Although these experiments suggest that thymus-dependent suppressor cells are involved in the nonspecific suppression of immunity, it is possible that specific suppressor cells are also generated in these infections. We are attempting to analyse in greater detail the mechanism by which the nonspecific suppressor cells act and their role in the pathogenesis of trypanosomiasis.

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Immunologic studies in contacts of osteosarcoma in humans and animals

AGENT(S) which could cause unrecognised or subclinical infections in otherwise healthy human household contacts of cancer patients may be detected by epidemiologic studies. Support for this hypothesis is provided by the reports of an increase in tumour specific humoral antibodies, as well as tumour specific cell mediated immunity[1–4] in the household contacts of patients with osteosarcoma, adenocarcinoma of the breast and other malignancies. Clear evidence for the validity of this conclusion has not been obtained. To test this hypothesis, we studied antibodies (both humoral and cellular) in the household contacts of a patient with osteosarcoma. In addition, in an animal model for human osteosarcoma, we characterised the sera and lymphocytes of hamster mothers (in close contact with their offspring) before and after induction of the tumour in their immunosuppressed offspring.

The humoral antibodies were tested by the indirect immunofluorescence test against tumour tissue imprints obtained from a patient (M.Mc.) with osteosarcoma of the femur. The membrane immunofluorescence test was performed with TE-85 cells (from an unrelated patient with osteosarcoma) maintained in tissue culture. Sera from five of the six household contacts of the patient (M.Mc.) showed positive reactions for humoral antibodies against both osteosarcoma imprints and TE-85 cells. Similar positive reactions were observed with the sera from four other (unrelated) patients with osteosarcoma and the patient's (M.Mc.) own serum for the presence of humoral antibodies. Only the serum of one of nine normal individuals showed positive immunofluorescence against both the tumour imprints and cells.

In the animal experiments, sea from mothers of tumour-bearing hamsters were subjected to the immunofluorescence test before the injection of TE-85-M-MSV cells into anti-lymphocyte serum (ALS) treated newborn hamsters. The sera from the hamster mothers were again tested on 5 and 20 d after inoculation of TE-85-M-MSV cells into the offspring (the tumours developed in the offspring 10–14 d after inoculation). Sera from eight of the 10 hamster mothers were positive for antibodies (against tumour imprints and TE-85 cells) on d 20 after the inoculation of the cells into the newborn hamsters. The sera from the same hamster mothers showed a negative reaction for antibodies on the day of the injection of cells and on d 5 after injection. None of the sera from the hamster mothers in the control group showed positive immunofluorescence.

Tumour specific antibodies were demonstrable against osteosarcoma imprints (by immunofluorescence) in the sera of 83% of the tumour-bearing newborn hamsters. 87% of the sera from these animals reacted positively against TE-85 cells. In the control group of newborn hamsters, only 4% of the animals had sera which reacted positively against osteosarcoma imprints and TE-85 cells (Table 1).

For the cell mediated immunity, the lymphocyte microcytotoxicity test was performed as described by Singh *et al.*[5]. The TE-85 cells maintained in tissue culture were used as target cells. Table 2 shows the percentage of inhibition of the target cells by the lymphocytes from the household contacts of the patient (M.Mc.), the patients with osteosarcoma and normal individuals. Significant inhibition by the lymphocytes was observed in five of the six same household contacts, all of the patients with osteosarcoma and one of the nine normal individuals.

In the animal model, the same eight hamster mothers had lymphocytes that exhibited significant cytotoxicity against TE-85 cells at d 20. The lymphocytes from the same mothers exhibited minimal cytotoxicity before the inoculation of cells into newborn hamsters and on d 5 after the cell

Table 1 Tumour-specific antibodies in tumour-bearing newborn hamsters

		Osteosarcoma imprints		TE-85 cells	
	No. of hamsters	Positive sera* per hamster test	%	Positive sera* per hamster test	%
Experimental group	72	60/72	83	63/72	87
Control group	23	1/23	4	1/23	4

For osteosarcoma imprints a standard indirect immunofluorescence test was performed against fixed sections. For membrane immunofluorescent 3 × 10^5 cells ml^{-1} were suspended in the test serum 1:10 dilution and incubated at 4 °C for 30 min. Fluorescent conjugated goat anti-hamster γ-globulin 1:10 dilution were used.

*Sera were drawn on d 20 after the injection of TE-85-M-MSV cells. Controls were normal newborn hamsters (no exposure to TE-85-M-MSV cells).

Table 2 Cytotoxicity of lymphocytes from osteosarcoma patients and their contacts

Experiment no.	Source of lymphocytes		No. of target cells per well 48 h after exposure to: Medium only	Medium plus lymphocytes	% Inhibition of target cells
1	RL	(patient)	499.4 ± 21.6	189.8 ± 15.3	62.0 ± 4.99*
2	US	(patient)	715.0 ± 28.6	375.4 ± 21.7	55.9 ± 3.23*
	RO	(patient)		208.8 ± 17.6	70.8 ± 5.96*
3	LM	(patient)	610.0 ± 28.1	128.1 ± 09.5	79.0 ± 5.85*
4	MMC	(patient)	842.3 ± 12.0	433.3 ± 0.91	48.5 ± 1.02*
5	JMC	(HHC)		202.6 ± 11.0	73.1 ± 3.99*
	CMC	(HHC)	755.0 ± 06.4	136.0 ± 05.8	81.9 ± 3.54*
	KMC	(HHC)		79.3 ± 20.9	89.4 ± 23.6*
	BMC	(HHC)		183.3 ± 05.4	75.7 ± 2.24*
6	JMC	(HHC)	825.3 ± 11.5	590.6 ± 07.4	28.4 ± 0.35*
	TMC	(HHC)		860.6 ± 08.9	2.2 ± 0.02
7	KT	(N)	568.0 ± 25.4	521.0 ± 16.7	8.2 ± 0.26
8	IS	(N)	502.6 ± 26.7	451.9 ± 22.9	10.1 ± 0.51
9	JC	(N)	439.4 ± 21.6	457.5 ± 19.8	8.5 ± 0.36
10	GS	(N)	451.0 ± 21.8	390.6 ± 23.2	13.2 ± 0.78
11	NK	(N)	610.0 ± 28.1	353.8 ± 25.6	42.1 ± 3.05*
12	DR	(N)	500.2 ± 13.9	455.0 ± 11.8	9.6 ± 0.24
13	CD	(N)	402.1 ± 25.6	368.4 ± 21.8	8.4 ± 0.48
14	MC	(N)	842.3 ± 12.0	811.0 ± 14.9	3.6 ± 0.06
15	AB	(N)	451.1 ± 21.8	462.0 ± 21.1	7.6 ± 0.34

Subjects were household contacts of patient MMC (HHC), normal individuals unrelated to patient MMC (N), and a normal control who had worked with osteosarcoma cells in the laboratory (NK). Lymphocytes from patients and normal controls were used as effector cells and TE-85 cells were the target cells for the microcytotoxicity test.

*Statistically significant, $P < 0.05$.

†Mean ± s.e.m.

injection. The lymphocytes from control animals showed no significant cytotoxic effect against TE-85 cells.

The results of the lymphocyte microcytotoxicity test, on tumour-bearing hamsters on d 20 (Table 3), showed that 75% of the animals showed significant cytotoxic effect against the target cells. In the control group, only 4% of the animals showed a significant reaction.

Hellström and Hellström[3] reported cellular immunity directed against neuroblastoma in the mothers of children with that tumour. Byers *et al.*[4] demonstrated that household contacts of osteosarcoma patients and patients with adenocarcinoma of the breast had specific immunity against the tumour type with which there had been contact. Furthermore, the range of cytotoxicity values produced by lymphocytes from the household contacts was significantly higher than that of the normal population. In the present investigation, all the osteosarcoma patients undergoing the lymphocyte microcytotoxicity test had higher cell mediated immunity against TE-85 cells than normal individuals. In the animal study, 80% of mothers of the tumour-bearing hamsters developed higher cell mediated immunity than the mothers in the control groups.

Morton and Malgren[6] interpreted the transformation of embryo fibroblasts (in tissue culture) as evidence for a transmissible agent (either horizontal or vertical) in patients with the tumours. An electron microscopic finding of occasional particles resembling murine leukaemia virus (in some biopsy specimens and cells of tissue cultures derived from osteosarcoma) were considered to indicate the presence of a viral antigen or virus-induced tumour antigen in the tested tumour.

In our animal model there was an 80% incidence of humoral antibodies against osteosarcoma imprints and the TE-85 cells in the sera of mothers of tumour-bearing newborn hamsters. This was demonstrated only after the tumours developed and not before tumour development or during tumour formation. The same was true of the cell mediated immunity. The production of antibodies against osteosarcoma in the animal model suggests a change in the immune mechanisms of the hamster mothers in close contact with tumour-bearing animals, and indicates that such a change was related to the development of osteosarcoma in the animal. Our results support the hypothesis that an agent is responsible for transmission of horizontal immunity to close contacts of patients with spontaneously arising osteosarcoma, and suggest that an infectious agent may be an aetiologic factor in human osteosarcoma.

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Table 3 Lymphocyte cytotoxicity in mothers of newborn hamsters 20 d after injection of TE-85-M-MSV cells in newborns

Source of lymphocytes, cage no.	% Target cell inhibition
34	29.09 ± 4.24
16	63.73 ± 11.52
33	23.00 ± 9.86
29	34.03 ± 8.44
1	33.31 ± 9.67
2	46.41 ± 7.71
4	30.69 ± 8.83
6	60.16 ± 12.62

Lymphocytes from hamster mothers at d 20 were used as effector cells against target cells (TE-85) in a ratio of 1:25 for the microcytotoxicity test. Samples for controls were taken from mothers of newborn hamsters which had been injected with saline instead of TE-85-M-MSV cells and showed no significant inhibition.

$P < 0.05$, statistically significant.

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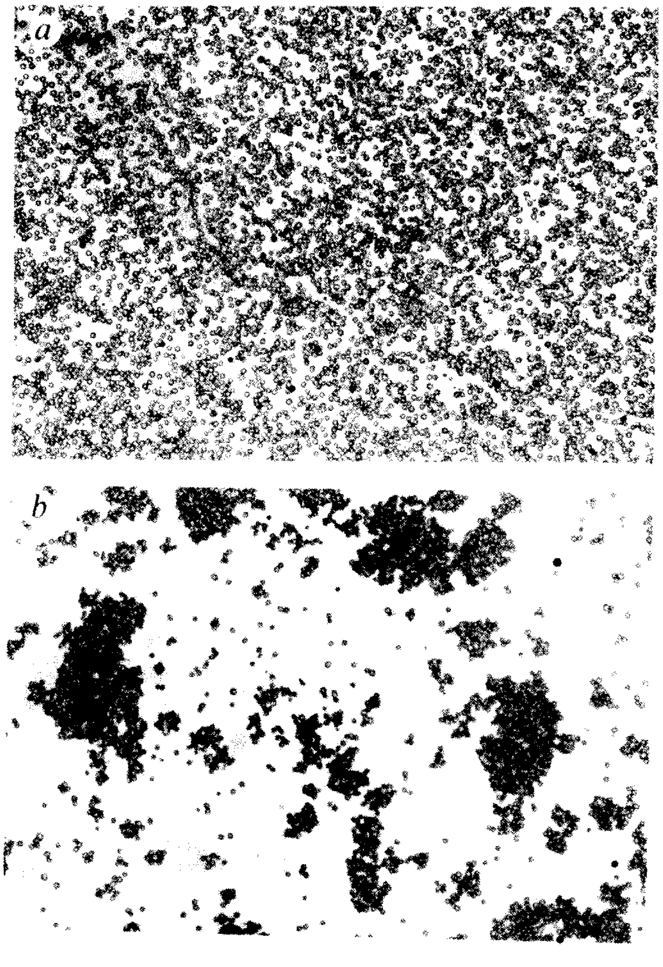
Requirement for magnesium influx in activation of alveolar macrophages mediated by ionophore A23187

NORMAL antibacterial activity in the lung is largely dependent on alveolar macrophage function[1]. Products of antigen-stimulated, sensitised lymphocytes can activate alveolar macrophages, which then exhibit greater adherence, phagocytosis and nonspecific bactericidal capacity than normal[2]. Although the molecular basis of this activation is unknown, influx or intracellular redistribution of divalent cations (Ca^{2+} and Mg^{2+}) has been implicated in other cell types[3-10]. We have therefore examined the effect on alveolar macrophage function of exposure to the ionophore A23187, which selectively binds divalent cations at neutral *p*H and passively transfers them across biological membranes[11,12].

Monolayers of normal rabbit alveolar macrophages were exposed for 1 h to tissue culture medium containing 5 μM A23187 or to normal medium alone. Cells were then washed and reincubated in fresh medium without ionophore for a further 18–72 h. Macrophage function (adherence to culture dishes, bactericidal activity and acid phosphatase content) was then assayed[2,14].

Macrophages studied 18–24 h after exposure to ionophore were similar in their properties to control macrophages, but cells left in normal medium for 40–72 h after 1 h of contact with ionophore, showed evidence of activation (Table 1). They increased in cell agglutinability or clumping (Fig. 1), adherence to tissue culture dishes and bactericidal activity against *Listeria monocytogenes* compared with normal cells (Table 1). The lysosomal hydrolytic enzyme, acid phosphatase, was also increased in the cells stimulated by ionophore.

Fig. 1 Cellular aggregation or agglutinability was perhaps the most striking manifestation of ionophore exposure. It is illustrated by photomicrographs of macrophage monolayers exposed for 1 h to A23187-containing or control media, followed by washing and reincubation in fresh medium without ionophore for 48 h. *a*, The control is characterised by a uniform layer of macrophages; *b*, marked cellular agglutination and clumping is seen after exposure to A23187 (×23.5).



We considered the possibility that A23187, a *Streptomyces* antibiotic[11], contributes to the intracellular killing of ingested *L. monocytogenes*. This was thought unlikely because macrophage bactericidal activity was normal at 18–24 h but had increased at 40–72 h after exposure to ionophore. Direct testing revealed that A23187 at concentrations up to 5 μM (2.6 μg ml^{-1}) in medium TC 199 with normal rabbit serum (NRS) failed to kill *L. monocytogenes* during a 2-h incubation (the time used in macrophage bactericidal assays).

Table 1 Function of alveolar macrophages after exposure to control or A23187-containing media

Experimental group	% Cell adherence*	% Acid phosphatase content†	% Intracellular bacterial survival (2 h)‡
Control	—	—	73.1 ± 8.4§ (23)
A23187	209.5 ± 30.8¶ (15)	119.6 ± 3.2 (52)	26.2 ± 3.1** (15)

*Calculated as

$$\frac{\text{no. of macrophages on dishes exposed to A23187}}{\text{no. of macrophages on control dishes}} \times 100$$

†Calculated as

$$\frac{\mu\text{mol product/mg cell protein-macrophages exposed to A23187}}{\mu\text{mol product/mg cell protein-control macrophages}} \times 100$$

‡Calculated as

$$\frac{\text{viable intracellular bacteria at 2 h}}{\text{viable intracellular bacteria at 0 time}} \times 100$$

§Mean ± s.e.m. Number of experiments in parentheses.

¶Significantly different from control ($P < 0.005$).

Significantly different from control ($P < 0.001$).

**Significantly different from control ($P < 0.001$).

Cells collected by lavage from the lower respiratory tract of male New Zealand rabbits were washed and then diluted in tissue culture medium 199 containing 15% normal heat-inactivated rabbit serum (TC 199-NRS), placed in tissue culture dishes and incubated for 4 h at 37 °C in 95% air–5% CO_2 (refs 2, 13). Nonadherent cells were then removed, and the macrophages were exposed to 5 μM A23187 in TC 199-NRS or to normal medium alone for 1 h. Monolayers were washed and reincubated in fresh TC 199-NRS without ionophore for 40-72 h before studies of macrophage function. At the conclusion of this incubation, determinations of adherence to tissue culture dishes and bactericidal activity against *Listeria monocytogenes* were performed as previously described[2]. For the acid phosphatase assay, macrophages were lysed in 0.1% Triton X-100 (ref. 14). *p*-Nitrophenyl phosphate sodium served as the substrate.

To investigate the need for specific divalent cations in ionophore-mediated activation macrophages were exposed to A23187 in TC 199–NRS or in salt solutions with various concentrations of Ca^{2+} and Mg^{2+} (Table 2). Ionophore in the presence of physiological concentrations of Ca^{2+} and Mg^{2+} (tissue culture medium and balanced salt solution), or physiological Mg^{2+} with markedly decreased Ca^{2+} (10^{-5} M), stimulated acid phosphatase activity. In contrast, exposure of cells to A23187 in a salt solution containing physiological Ca^{2+} and decreased Mg^{2+} (10^{-5} M) produced no increase in enzyme activity. The addition of 10 mM MgEGTA to A23187-containing TC 199–NRS, providing a physiological concentration of Mg^{2+} (3×10^{-3} M) and a marked reduction in Ca^{2+} content (10^{-10} M)[15], abolished the activating effect of the ionophore.

The cell-mediated immune response produces marked changes (activation) in systemic macrophages. Compared with normal macrophages, activated cells are larger, morphologically more complex, and show greater adherence, ingestion, nonspecific bactericidal activity, oxygen uptake,

Table 2 Acid phosphatase content of alveolar macrophages after exposure to A23187-containing solutions

Experimental group	Divalent cations (molar concentration) Mg^{2+}	Ca^{2+}	% Acid phosphatase content*
TC 199-NRS	1×10^{-3}	1.8×10^{-3}	
Control			100
A23187			119.6 ± 3.2†‡ (52)
Balanced salt solution	1×10^{-3}	1.8×10^{-3}	
Control			100
A23187			110.7 ± 3.7§ (17)
Low Ca^{2+} salt solution	1×10^{-3}	1×10^{-5}	
Control			100
A23187			114.5 ± 6.9¶ (22)
Low Mg^{2+} salt solution	1×10^{-5}	1.8×10^{-3}	
Control			100
A23187			95.2 ± 2.7 (12)

The experimental procedure was as described in Table 1, except that in the experiments indicated the exposure of macrophages to ionophore took place in specified salt solutions rather than in TC 199-NRS. These salt solutions were Earle's or Hanks' balanced salt solution (BSS) or 'calcium-and magnesium-free' BSS, to which Ca^{2+} or Mg^{2+} was added in the indicated concentration. After brief exposure to A23187-containing or control medium, cells were washed and reincubated in fresh culture medium (with normal Ca^{2+} and Mg^{2+} and without ionophore) for 48–72 h before assay.

*Calculated as $\frac{\mu\text{mol product per mg cell protein-macrophages exposed to A23187}}{\mu\text{mol product per mg cell protein-control macrophages}} \times 100$

†Mean ± s.e.m. Number of experiments in parentheses.
‡Significantly different from control ($P < 0.001$).
§Significantly different from control ($P < 0.025$).
¶Significantly different from control ($P < 0.05$).

glucose oxidation and content of hydrolytic enzyme[16]. Acquired resistance to systemic infection with many intracellular organisms depends on these changes. Our demonstration that lymphokines activate alveolar macrophages has implied that this protective mechanism is also intact in the lung[2]. Certain common features of activation in other cell types suggest a potential mechanism for the lymphokine-mediated process in macrophages. Influx or intracellular redistribution of divalent cations has been implicated as the initiating event in activation of various cells, including lymphocytes, fibroblasts and sea urchin eggs[3–10]. Much of the evidence for the importance of divalent cations (especially that ascribed to Ca^{2+}) in cellular activation was obtained using A2318 (refs 3, 5–7).

There are at least three possible mechanisms by which A23187 and divalent cations might initiate cell activation. First, a requirement for Ca^{2+} may be related to induced increases in intracellular cyclic GMP (refs 10, 17, 18). Increases in cyclic GMP concentration (and/or decreases in the ratio of cyclic AMP : cyclic GMP) are associated with proliferation or activation in lymphocytes and fibroblasts[19–23]. Second, the postulated importance of Mg^{2+} in the stimulation of cell proliferation (chick embryo fibroblasts) could be related to the requirement of this cation for transphosphorylation reactions. Thus increasing the concentration of intracellular free Mg^{2+} may activate cellular metabolism and growth[9,24]. Third, A23187 may initiate activation by producing a charge-neutral exchange of $2\,H^+$ for each divalent cation transported across the plasma cell membrane[11,12,25]. This H^+ efflux (exchange of $2\,H^+$ for each Ca^{2+} or Mg^{2+}) may lead to an increase in intracellular cytoplasmic pH; such a change in intracellular pH has been reported to initiate activation of sea urchin eggs[26].

In view of these possibilities we examined the requirements for initiation of alveolar macrophage activation. We found that to activate normal alveolar macrophages A23187 needs both near-physiological concentrations of extracellular Mg^{2+} and very low concentrations (between 10^{-5} and 10^{-10} M) of Ca^{2+}. Thus Ca^{2+} influx alone is not the initiating event for A23187-induced macrophage activation Our results indicate that Mg^{2+} is the major extracellular divalent cation required at the onset of activation. These results apparently contrast with reports that Ca^{2+} influx is the initial event in ionophore-mediated activation of other cells[3,5–7]. It is also true, however, that in some of these studies Ca^{2+} influx was merely assumed to be the basis of the A23187 effect, and other possibilities were not excluded (such as a role for Mg^{2+}). In any case the need for Mg^{2+} in the activation of alveolar macrophages is clear, although the mechanism involved is not.

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Interrelationship of cyclic nucleotides and anaphylactic reactions

THE mode of action of several anti-allergic drugs, which are structurally diverse, has been studied by comparing their inhibition of cyclic nucleotide phosphodiesterase with inhibition of anaphylaxis. The cyclic nucleotide phosphodiesterase activities were measured in microsomal preparations of both human and sheep lung, and the anaphylaxis was observed *in vitro* in passively sensitised human lung and in rats *in vivo* (passive cutaneous anaphylaxis (PCA)). A significant correlation has been found between the inhibition of anaphylaxis and the ratio of the inhibition of hydrolysis of cyclic AMP to the inhibition of hydrolysis of cyclic GMP. A less significant correlation was also found between inhibition of rat PCA and inhibition of hydrolysis of cyclic GMP.

The studies of Austen *et al.*[1-3] have shown that inhibition of anaphylactic release of histamine from human lung tissue can be associated with raised tissue levels of cyclic AMP. Lichtenstein and Margolis[4] have shown that methylxanthines inhibit the antigen-induced release of histamine from human leukocytes and it has been assumed that this is due to their ability to inhibit cyclic AMP hydrolysis[5]. A relationship has been suggested[6-8] between the activity of anti-allergic compounds and their ability to inhibit cyclic 3′,5′-nucleotide phosphodiesterases. Austen *et al.*[2] have also suggested that, in contrast to the effects of cyclic AMP, raised levels of cyclic GMP can potentiate the antigen-induced release of histamine from human lung tissue. If, as is believed, the effect of increasing cyclic AMP levels is to inhibit the release of histamine and of raising cyclic GMP levels is to potentiate histamine release[1-3], the observed pharmacological effect of any agent inhibiting the hydrolysis of both nucleotides is likely to be related to the resultant change in the balance of the two nucleotide levels.

We have therefore investigated the ability of known and novel anti-allergic compounds to inhibit cyclic AMP and cyclic GMP hydrolysis and to inhibit anaphylaxis. Table 1 shows the pharmacological potency of twenty-five compounds in inhibiting anaphylaxis in passively sensitised human lung tissue and/or rat skin. Table 1 also shows the concentrations of the various compounds required to inhibit the hydrolysis of both cyclic nucleotides by 50% (I_{50}) using a microsomal preparation from human lung, and the inhibition constants (K_{I}) using a microsomal preparation from sheep lung. Also shown are the ratios of I_{50} (human lung) and K_{I} (sheep lung) for the hydrolysis of cyclic AMP to that of cyclic GMP. Table 2 shows the regression analyses of the relevant terms. A very significant correlation exists between the ability to inhibit anaphylactic reactions and the ratio of I_{50} cyclic AMP to I_{50} cyclic GMP or K_{I} cyclic AMP to K_{I} cyclic GMP. Figure 1 illustrates one of these relationships, that is that between potency of compounds in the human lung and the I_{50} ratio determined in human lung microsomes. This result indicates that com-

Table 1 Inhibition of anaphylaxis and phosphodiesterase activity

Inhibitor*	Pharmacological potency: relative to DSG = 1.00 Human lung	Rat PCA	Human lung I_{50} cyclic GMP (M)	I_{50} cyclic AMP (M)	I_{50} cyclic AMP / I_{50} cyclic GMP	Sheep lung K_{I} cyclic AMP (M)	K_{I} cyclic GMP (M)	K_{I} cyclic AMP / K_{I} cyclic GMP
Theophylline[15]	0.04	0.01	5.60×10^{-4}	1.90×10^{-3}	0.29	5.70×10^{-4}	4.85×10^{-4}	1.18
Papaverine[16]	0.12	0.07	4.50×10^{-5}	5.20×10^{-5}	0.87	2.33×10^{-5}	2.97×10^{-5}	0.78
M+B 21231(40)[18]†	0.16	0.47	5.70×10^{-4}	6.40×10^{-4}	0.89	2.91×10^{-3}	5.31×10^{-4}	5.48
M+B 21703(19)[18]†	0.22	6.23	4.01×10^{-5}	4.45×10^{-5}	0.90	1.17×10^{-4}	3.57×10^{-5}	3.28
Sc 2964[15]	0.27	0.04	2.62×10^{-5}	2.85×10^{-5}	0.92	1.11×10^{-4}	4.06×10^{-5}	2.73
M+B 23268(18)[17]†	0.32	0.29	3.10×10^{-4}	1.67×10^{-4}	1.86	3.70×10^{-4}	2.84×10^{-5}	13.03
Indomethacin[28]	0.74	0.01	6.10×10^{-5}	4.00×10^{-5}	1.50	6.86×10^{-4}	1.67×10^{-4}	4.11
Ketoprofen[29]	0.82	0.01	5.60×10^{-3}	2.15×10^{-3}	2.60	4.96×10^{-3}	5.57×10^{-3}	0.89
Disodium cromoglycate[19]	1.00	1.00	4.80×10^{-3}	6.20×10^{-3}	0.77	4.12×10^{-3}	5.46×10^{-4}	7.55
M+B 21378(7)[18]†	1.30	4.75	1.00×10^{-3}	1.90×10^{-4}	5.26	3.01×10^{-3}	5.83×10^{-4}	5.16
Sch. 15280[20]	1.55	—	3.85×10^{-4}	1.80×10^{-3}	0.21	—	—	—
RO 20-1724/1(16)[21]†	2.55	—	1.70×10^{-3}	1.68×10^{-3}	1.01	—	—	—
SQ20009[22]	3.45	—	2.15×10^{-4}	3.55×10^{-5}	6.42	5.99×10^{-5}	1.20×10^{-5}	5.00
M+B 22106(22)[17]†	4.67	5.33	1.60×10^{-4}	1.10×10^{-4}	1.46	1.42×10^{-3}	2.62×10^{-4}	5.42
A+H 7079[24]	8.45	5.74	1.60×10^{-5}	2.83×10^{-6}	5.65	3.44×10^{-3}	2.41×10^{-4}	14.27
ICI 58301[25]	9.00	0.05	6.75×10^{-4}	2.55×10^{-4}	2.65	7.17×10^{-3}	2.17×10^{-3}	3.30
Doxantrazole[7]	13.20	0.24	—	—	—	5.88×10^{-5}	2.94×10^{-5}	2.00
M+B 25565[26]	14.30	792.97	2.95×10^{-4}	1.57×10^{-5}	18.80	4.27×10^{-4}	5.13×10^{-6}	83.24
ICI 74917[5]	15.30	69.53	—	—	—	4.13×10^{-4}	3.53×10^{-5}	11.70
M+B 23231(27)[17]†	16.50	5.02	1.25×10^{-4}	2.57×10^{-5}	4.86	1.61×10^{-3}	1.98×10^{-4}	8.13
M+B 22948[27]	17.40	10.59	1.25×10^{-4}	7.95×10^{-6}	15.70	1.66×10^{-4}	2.01×10^{-5}	8.26
A+H 7725[24]	24.00	6.29	2.30×10^{-3}	2.42×10^{-4}	9.50	5.06×10^{-3}	3.17×10^{-4}	16.00
BRL 10833(87)[23]†	42.00	9.70	8.50×10^{-3}	7.90×10^{-4}	10.80	2.46×10^{-3}	1.49×10^{-4}	16.50
ICI 63197[25]	—	0.02	—	—	—	7.66×10^{-5}	1.07×10^{-3}	0.07

Inhibition of anaphylaxis in the human lung was measured using macroscopically normal tissue specimens obtained at lobectomy. Lung parenchyma was prepared and sensitised as described before[9]. The effects of drugs on the release of histamine were determined by an incubation method[10], using an extract of *D. farinae* as antigen. The drugs were added immediately before challenge. Histamine was assayed fluorimetrically[11]. Potency of drugs relative to disodium cromoglycate was calculated by comparing the molar concentrations required to produce equivalent inhibition of histamine release (30–40%). In the rat, passive sensitisation of skin areas was produced by intradermal injection of *Nippostrongylus brasiliensis* antiserum. After 48 h rats were challenged intravenously with *N. brasiliensis* extract. The compounds were administered intravenously immediately before challenge. The effective dose of each compound was measured as the minimal concentration to give 100% inhibition of the cutaneous reaction (ID_{100}) in two rats. Potency of drugs relative to disodium cromoglycate was calculated as the ratio of ID_{100} disodium cromoglycate (M) to ID_{100} test drug (M). In preparation for phosphodiesterase assay human lung was chopped with a McIlwain chopper and then ground with sand. The resulting homogenate was centrifuged to obtain the 100,000*g* pellet which was resuspended in 0.1 M Tris buffer, *p*H 7.4. The assay system (0.2 ml) contained 0.16 μM ^{3}H-cyclic nucleotide, 4.5 mM $MgSO_4$ and microsomes (0.5–1.0 μg per ml protein) in Tris buffer 0.1 M, *p*H 7.4. Addition of 5′-nucleotidase was found to be unnecessary. Enzyme activity was measured after 20 min at 37 °C as the rate of formation of tritiated products[12]. Each compound was tested twice using five concentrations in duplicate. The I_{50}s were obtained graphically and averaged. Sheep lung microsomal fraction was prepared as for human lung and the inhibition of phosphodiesterase activity was measured as above, but using the modification described by Boudreau and Drummond[13]. This allows for the quantitative recovery of adenosine, guanosine and their metabolites, which are produced when assaying crude enzyme preparations or tissue homogenates. After the discovery of a calcium-dependent protein activator for bovine brain phosphodiesterase[14], 0.5 mM calcium chloride was added to the assay medium to maximise phosphodiesterase activation. Substrate concentration was varied from 3.33 to 20 μM. Values of K_{I} were calculated by double reciprocal plot, using not less than four independent determinations.

*Suffixes refer to the publication in which the compounds are described.

†A compound with a number in parentheses is exemplified by that number in patent claim or paper.

Table 2 Multiple regression analysis

Variables 1	Variables 2	No. of observations	Slope	Intercept	Student's t test	Correlation coefficient R	P
log. pharm. pot. (lung)	log. I_{50} cyclic GMP	21	−0.26	−0.75	1.26	0.278	NS
log. pharm. pot. (rat)	log. I_{50} cyclic GMP	18	−0.73	−2.98	1.94	0.436	<0.1
log. pharm. pot. (lung)	log. I_{50} cyclic AMP	21	0.24	1.09	0.97	0.217	NS
log. pharm. pot. (rat)	log. I_{50} cyclic AMP	18	−0.14	−0.64	0.31	0.078	NS
log. pharm. pot. (lung)	log. K_1 cyclic GMP	21	−0.16	−0.27	0.59	0.134	NS
log. pharm. pot. (rat)	log. K_1 cyclic GMP	21	−1.11	−4.39	2.99	0.567	<0.01
log. pharm. pot. (lung)	log. K_1 cyclic AMP	21	0.30	1.28	1.11	0.247	NS
log. pharm. pot. (rat)	log. K_1 cyclic AMP	21	0.16	0.34	0.34	0.078	NS
log. pharm. pot. (lung)	log. R (K_1)*	21	1.23	−0.53	3.86	0.663	<0.001
log. pharm. pot. (rat)	log. R (K_1)*	21	1.82	−1.34	5.24	0.769	<0.001
log. pharm. pot. (lung)	log. R (I_{50})*	21	1.25	−0.19	5.68	0.794	<0.001
log. pharm. pot. (rat)	log. R (I_{50})*	18	1.84	−0.89	3.30	0.637	<0.01

The data in Table 1 were submitted to multiple regression analysis using a DEC System PDP—10 computer. NS, not significant at the 0.1 level.

*$R(I_{50}) = I_{50}$ cyclic AMP/I_{50} cyclic GMP human lung; $R(K_1) = K_1$ cyclic AMP/K_1 cyclic GMP sheep lung.

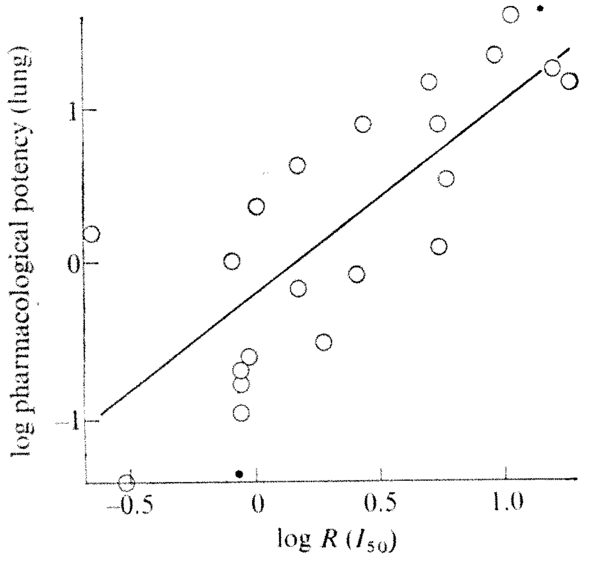
pounds which are more potent inhibitors of the anaphylactic reaction inhibit the hydrolysis of cyclic GMP more effectively than that of cyclic AMP. We find that the correlation does not hold for the supernatant enzyme activity from human lung (unpublished work of C.J.C.). This suggests that the effect of the cyclic nucleotides takes place in a membrane environment.

Relationships of lesser significance are obtained between inhibition of cyclic GMP hydrolysis and pharmacological potency in the rat. These results indicate that although the balance between the two cyclic nucleotides seems to be critical in determining the anti-allergic activity of a compound, cyclic GMP seems to be the more important factor in the rat PCA test.

In some cases it seems to be difficult to reconcile the drug concentrations effective in inhibiting histamine release from human lung fragments with those which inhibit human lung microsomal phosphodiesterase. The apparent discrepancy could possibly be related to the lipophilic properties of the drugs concerned and the sites of the enzymes involved. Direct measurement of drug-induced changes in cyclic nucleotide levels within the membranes of target cells would provide a better understanding of their interrelationship. Further work is planned in an attempt to elucidate these points.

Cyclic AMP and cyclic GMP have been suggested to have antagonistic actions as intracellular messengers and regulators in certain biological systems[30]. There have been several reports of control systems of this type[31–33]. It seems likely that such a mechanism is operating here and that the selectivity of the inhibitor between the two cyclic nucleotide phosphodiesterases governs the biological activity. The implication of our results is that histamine release is reduced more effectively when cyclic GMP levels are increased with respect to cyclic AMP levels. This result is in opposition to the concept discussed by Austen *et al.*[1–3], but they present only circumstantial evidence, linking increased cyclic GMP levels with increased histamine release. No workers, as far as we know, have measured the levels of both nucleotides in human lung simultaneously. It is interesting that McElroy and Philip[34] also find that the ratio of K_1s but not the individual K_1 for each cyclic nucleotide correlates with inhibition of ADP-induced aggregation in platelets.

Fig. 1 The logarithm of the pharmacological potency plotted against the logarithm of the I_{50} ratio. The straight line was obtained by regression analysis. Data are from human lung.



The fact that highly significant correlations were obtained between selective inhibition of phosphodiesterase activity and pharmacological potency in *in vitro* and *in vivo* tests, involving different species and different tissues, may reflect a basic common mechanism of fundamental importance in allergic reactions.

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Sperm-associated sialyltransferase activity

FERTILISATION begins with a specific adhesion between sperm and egg and culminates with the fusion of the male and female pronuclei. In mammals, sperm seem to recognise the zona pellucida surrounding the egg[1]. This adhesive interaction is the first of many that will take place during subsequent embryogenesis. In molecular terms, these later cell–cell interactions are not generally understood, and the mechanism of sperm–egg recognition is unknown. Conceptually, advances in this area have been rare since Lillie presented the fertilisin hypothesis, and even this has been questioned[2]. There is evidence to support a model for intercellular recognition that utilises the molecular complementarity inherent in cell surface glycosyltransferases and their respective acceptor molecules[3,4]. The transferases catalyse the addition of sugars, from sugar nucleotides, to the non-reducing termini of saccharide acceptors. The enzymes are highly specific for both the donor sugar nucleotide and the acceptor, usually an oligosaccharide. For example, sialyltransferases are the class of glycosyltransferases that transfer sialic acid (*N*-acetylneuraminic acid) from cytidine monophosphate-*N*-acetylneuraminic acid (CMPNAN) to either glycoprotein, glycolipid, or free oligosaccharide acceptors[4]. Glycosyltransferases have been reported on the external surfaces of many different cell types and, in some cases, enzyme–substrate complexes between the transferases and their acceptors may be instrumental in the cellular recognition that occurs (reviewed in ref. 5). We report here a study of mouse sperm–egg interactions in terms of the transferase–acceptor hypothesis.

CMPNAN : asialofetuin sialyltransferase activity was associated with intact sperm, although no endogenous acceptors for the enzyme were detected. Autoradiographic assays supported the absence of endogenous surface sialyltransferase activity on intact sperm, but showed moderate activity on mouse ova from C57Bl females superovulated by injection of 10 U of gonadotropin from pregnant mare serum (Sigma) and, after 48 h, by injection of 10 U of human chorionic gonadotropin (Sigma). Ova were collected from the dissected uterine ampullae 16 h after injection of the human chorionic gonadotropin. When sperm and ova were incubated together with the sialic acid donor, additional dense grain patches were found on the ova surfaces. These accumulations were often associated with penetrated sperm (Fig. 3). Ova incubated identically, but without sperm, did not show these patches (Fig. 2).

Sperm were obtained from the cauda epididymis and vas deferens of mature male C57Bl mice by dissection and collection in a saline solution, *p*H 7.3, containing (in g $^{-1}$): NaCl, 7.13; glucose, 1.0; KCl, 0.4; phenol red, 0.004, and HEPES, 2.35. This sperm suspension was centrifuged once at 10*g* for 4 min to sediment tissue debris. The supernatant sperm suspension was then washed three times by centrifugation (500*g*, 6 min) and resuspended in the saline solution described above.

The first, 500*g* supernatant from the sperm preparation and the corresponding sperm pellet contained approximately equivalent levels of sialyltransferase activity toward the exogenous acceptor asialofetuin. This supernatant enzyme was probably derived from seminal fluid, because levels of activity decreased in the next two supernatants while the activity of the sperm pellet remained constant. The activity observed with intact sperm may result from a sialyltransferase that is initially in seminal fluid and, secondarily, adsorbed on to sperm rather than being an intrinsic part of the sperm plasma or acrosomal membrane. In the conditions described in the legend to Fig. 1, and with 10^6 sperm as the

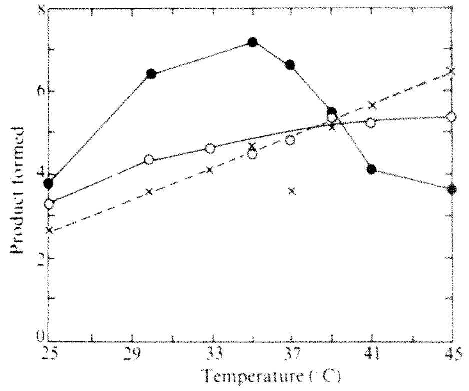


Fig. 1 Reaction rates of murine sperm enzymes as a function of temperature. Sialyltransferase activity toward asialofetuin, in pmol ^{3}H-sialic acid transferred per h per 10^6 sperm (●), acid phosphatase activity, in μmol *p*-nitrophenol formed per h per 10^6 sperm (○) and β-galactosidase activity, in μmol *p*-nitrophenol formed per h per 10^6 sperm (×). Asialofetuin was prepared from fetuin (Sigma) by mild acid hydrolysis in 0.1 N H_2SO_4 for 75 min at 80 °C. The hydrolysate was adjusted to *p*H 7.3 with 0.25 N NaOH and dialysed exhaustively in 0.01 M HEPES, *p*H 7.3, at 20 °C (5 ml hydrolysate against 3 × 700 ml dialysate over at least 24 h). Similarly, ^{3}H-NAN was obtained from CMP-^{3}H-NAN (New England Nuclear) by mild acid hydrolysis in 0.3 N HCl for 15 min at room temperature. The hydrolysate was neutralised with 0.3 N NaOH and assayed by descending paper chromatography on Whatman No. 1 paper with 95% ethanol and 1 M ammonium acetate (5:2) as a solvent. Quantitative cleavage of the CMP-^{3}H-NAN occurred in a concentrated solution (55 μl CMP-^{3}H-NAN at 2.33 Ci mmol^{-1} was desiccated and hydrolysed in 25 μl of HCl). For sialyltransferase assays 0.125 nmol of CMP-^{3}H-NAN, 1.25 mg asialofetuin and 25 μl of 10 mM $MgCl_2$ were desiccated in a small test tube (neither $MgCl_2$ nor $MnCl_2$ had any significant inhibitory or stimulatory affects). Reactions were initiated by adding 25 μl of the washed, intact sperm suspension to each tube before incubation at 35 °C. The reactions were stopped by the addition of 10 μl of ice-cold 0.25 M EDTA and by immediate chilling to 0 °C. Although cations were not required for the sialyltransferase reaction, the addition of EDTA consistently lowered electrophoretic background activities. The amount of ^{3}H-sialic acid transfer was determined by high voltage electrophoresis of 20 μl of the reaction mixture using methods described before[7]. Transfer to endogenous acceptors was determined by omitting asialofetuin from the reaction tubes. For acid phosphatase and β-galactosidase assays, 25 μl of the washed sperm suspension was added to 200 μl of a 50 mM citrate buffer, *p*H 5.1, containing either *p*-nitrophenyl phosphate or *p*-nitrophenyl β-D-galactopyranoside (both at 1 mg ml^{-1}, Sigma). After 1 h, the reactions were terminated by the addition of 1.5 ml ice-cold 0.02 M NaOH and the values of A_{400} were recorded and converted to μmol of product formed using a conversion factor of 0.245 Δ units per μmol free *p*-nitrophenol.

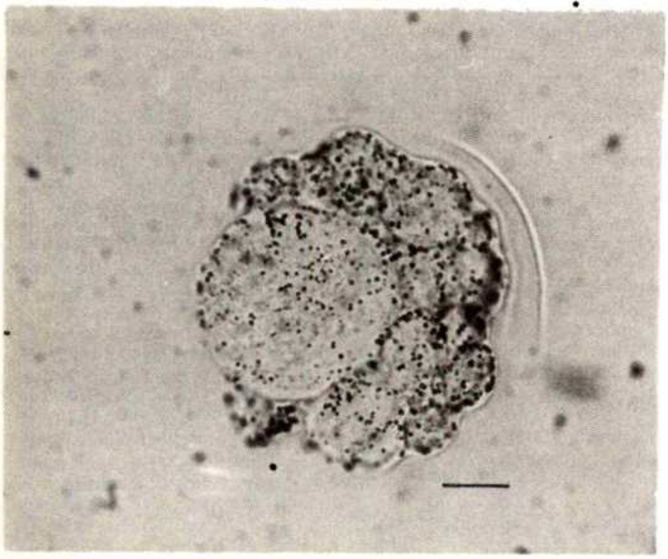
enzyme source, the rate of transfer was 4.5 pmol of sialic acid transferred per h at 35 °C. This rate was linear with time for at least 150 min. If asialofetuin was omitted from the reaction mixtures there was no detectable endogenous activity with washed sperm.

Figure 1 shows that the sialyltransferase activity on intact sperm was highest at 35 °C, the approximate temperature of the mouse testis[6], and that it decreased at higher or lower temperatures. This contrasts with sperm acid phosphatase and β-galactosidase, for which activity increases with temperature up to at least 45 °C.

When ova alone were incubated with CMP-^{3}H-NAN for 1 h and examined autoradiographically, they showed a definite endogenous incorporation that was significantly above background but diffusely distributed on the ova and surrounding cumulus cells (Fig. 2). Ten ova were assayed in this fashion in three separate experiments, and all showed the same pattern. By contrast, when 12 ova, in three experiments, were incubated with sperm for 1 h, all the eggs contained at least one very dense patch of silver grains in addition to the diffuse pattern seen on all eggs. Figure 3*a* shows a brightfield micrograph of an egg incubated with sperm and processed autoradiographically. This egg has three, clear deposits of silver grains in which the density of the grains is much greater than that of the surrounding area. The same egg is shown under phase contrast in Fig. 3*b*. Two of the dense grain deposits (arrows) in Fig. 3*a* are clearly associated with sperm that have penetrated the egg and whose tails are visible in phase contrast in Fig. 3*b* (arrows). The third patch of silver grains on the egg (leftmost edge of the egg in Fig. 3*a*) cannot be linked unambiguously to a single sperm although there are several possible candidates visible when the actual specimen is viewed at a number of focal planes in phase contrast. Grain accumulations are also seen on cumulus cells of eggs incubated with sperm (Fig. 3*a*) but not on cumulus cells of eggs incubated without sperm (Fig. 2). The localised, spatial correlation between grain patches and sperm indicate that the transfer is mediated by sperm-associated and not soluble enzyme activities.

In these incubations, hydrolysis of CMP-^{3}H-NAN did occur but the extent of hydrolysis did not exceed 17% of the input sugar nucleotide. Further, this level of free ^{3}H-NAN cannot account for any of the activities reported here because, when ^{3}H-NAN was substituted for CMP-^{3}H-NAN at identical concentrations (0.125 nmol per 25 μl) and specific activities (prepared as described in the legend to Fig. 1), there was no sialic acid transfer to asialofetuin by sperm.

Fig. 2 Brightfield micrograph of a mouse egg incubated in CMP-^{3}H-NAN and developed autoradiographically. There is a definite incorporation of radioactivity above background levels on the egg as well as on cumulus cells partially surrounding the egg, and this incorporation is distributed randomly. The bar represents 25 μm.



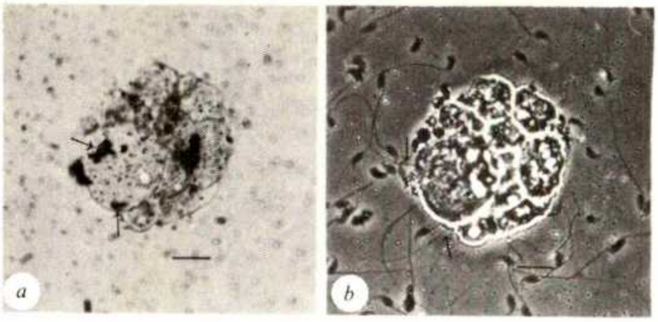


Fig. 3 Mouse egg incubated as in Fig. 2 but in the presence of washed sperm. *a*, Brightfield micrograph showing three dense accumulations of grains over the egg and several accumulations over the cumulus cells. These patches of grains are in addition to the general, evenly distributed incorporation seen in eggs incubated without sperm. Arrows indicate two grain patches on the egg. *b*, Phase contrast micrograph of the identical field shown in *a*. Here the sperm are easily seen. Arrows show the tails of sperm that have penetrated the egg in precisely those areas where two of the grain accumulations can be seen (arrows) in *a*. The bar represents 35 μm. Autoradiographic assays were performed using concentrations of CMP-^{3}H-NAN identical to those described in the legend to Fig. 1, except that the contents of the incubation tubes containing either ova alone or ova with washed sperm were first placed on a slide kept on a humidified chamber for the duration to the incubation. The reactions were stopped by gentle rinsing of the ova, or ova with sperm, with saline followed by fixation in 10% formalin. The slides were then stained lightly with haematoxylin and processed by standard autoradiographic techniques.

These data show that a CMPNAN : asialofetuin sialyltransferase is associated with mouse sperm and suggest that intact sperm can transfer sialic acid to acceptors on egg membranes. In the absence of added CMPNAN, it is possible that an enzyme–substrate complex would be more stable and could be one of the mechanisms whereby a sperm recognises and adheres to an egg. Although we have no data to suggest that the enzyme activity expressed by intact sperm results from intrinsic, as opposed to adsorbed, transferases, even an adsorbed enzyme could function in the suggested manner.

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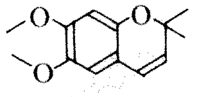
Precocene II inhibits juvenile hormone biosynthesis by cockroach corpora allata *in vitro*

BOWERS *et al.*[1] reported that a chromene derivative isolated from plants of the genus *Ageratum* interferes with the development of insects from several orders. They named the most active component of the plant extract precocene II (I), because exposure of young larval stages of test insects to the compound leads to precocious metamorphosis into unviable or moribund miniature adults. Also, exposure of adult females of several insect species causes sterility by preventing normal vitellogenic development of the oocytes, and in at least one case[1] this effect can be overcome by hormone replacement therapy with C_{16} juvenile hormone (C_{16}JH: (II)). These effects suggest that precocene II acts



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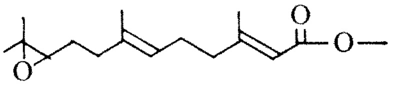

II

antagonistically to the natural JHs which are responsible for the proper suppression of metamorphosis in larval insects[2], and for the activation of ovarian growth and other reproductive phenomena in adult females of most insects[3]. Precocenes constitute a new type of insect growth regulator, and have been referred to as 'fourth generation insecticides'[1]. We envisaged two ways *a priori*, in which precocene might act in the whole animal: either by blocking the biosynthesis of JHs, or by blocking the action of natural JHs, that is, their safe transport through the haemolymph followed by effective action at the various target sites. We have concentrated on one aspect of the former possibility, and have investigated whether or not active corpora allata from adult females of the cockroach *Periplaneta americana* are directly inhibited by exposure to precocene II *in vitro*.

We used minor modifications of the quantitative assay for inhibitors of JH biosynthesis developed by Pratt and Finney[4], which is based on (1) the predictability of synthetic activity in corpora allata taken from adult mated females at specified times during the gonotrophic cycle, (2) the incorporation of label from methyl-^{14}C-methionine into C_{16}JH, and (3) the linearity of this incorporation for at least 5 h *in vitro*[6,7]. Because of natural increases in the reproduc- *in vitro* to concentrations of precocene II of 10^{-4} M and corpora allata are now maximally active 0.75 d after the mid-brown stage of ootheca formation[5], affording rates of C_{16}JH biosynthesis which range from 28 to 45 pmol per pair of glands per h. Figure 1 shows that spontaneously active corpora allata can be inhibited rapidly by exposure *in vitro* to concentrations of prococene II of 10^{-4} M and higher; moreover, the inhibition of C_{16}JH biosynthesis measured in these standard conditions is essentially complete at concentrations in excess of 10^{-3} M. The influence of precocene II on C_{16}JH biosynthesis was quantitatively indistinguishable from its effect on total ester production (methyl farnesoate + C_{16}JH). This level of inhibitory activity is approximately commensurate with that of some propynyl and methylenedioxyphenyl ethers previously examined but much lower than that of those methylenedioxyphenyl ethers which bear a strong structural resemblance to natural JHs and have intrinsic JH-mimicking activity[4]. There is no doubt that precocene II can act as a powerful direct inhibitor of the corpus allatum which suggests that analogous structures may have this activity at much lower concentrations, as is the case with methylenedioxyphenyl series.

That the observed level of methyl farnesoate in the glands treated with precocene II was reduced in proportion to the reduction in the rate of C_{16}JH biosynthesis might suggest that the site of action is earlier than the final epoxidative step in the biosynthetic pathway. Failure to accumulate methyl farnesoate in the presence of monooxygenase inhibitor, however, might also occur if internal feedback mechanisms prevented the overproduction of farnesenic acid and hence methyl farnesoate[4].

To investigate this possibility, we have made use of the fact that addition to the culture medium of the presumed precursor farnesenic acid results in a significant stimulation of the rate of C_{16}JH biosynthesis, regardless of the spontaneous level of activity of the glands. The exogenous ^{3}H-farnesenic acid is incorporated efficiently into the newly synthesised C_{16}JH (refs 8 and 9). Moreover, large quantities of methyl farnesoate accumulate within the glands in these conditions because of a limited epoxidative capacity. Apparently there is no rapid end-product inhibition of the *O*-methyl transferase. Thus by carrying out trials with precocene II in the presence and absence of exogenous farnesenic acid we were able to determine whether the inhibitor has an effect on JH biosynthesis subsequent to the point of entry of farnesenic acid into the biosynthetic pathway. The optimum concentration of farnesenic acid for stimulation of C_{16}JH biosynthesis by corprora allata from the locust *Schistocerca gregaria* is known to be 2–2.5 × 10^{5} M (ref. 10). Glands from *Periplaneta americana* respond optimally to slightly higher concentrations (unpublished results of G.E.P.) and so a concentration of 4–4.5 × 10^{-5} M was used. By killing animals between ovulation and ootheca formation (2.75 d after the mid-brown stage of the previous ootheca) we obtained corpora allata exhibiting low spontaneous rates of C_{16}JH biosynthesis (2–5 pmol per pair of glands per h)[5]; in the presence of optimal concentrations of farnesenic acid, these glands afforded rates of C_{16}JH biosynthesis of 40–55 pmol per pair of glands per h, and total esterification rates of 60–95 pmol per pair of glands per h.

Analysis of the isotope incorporation ratio in the C_{16}JH indicated that in these 'stimulated' conditions, 91–98% of

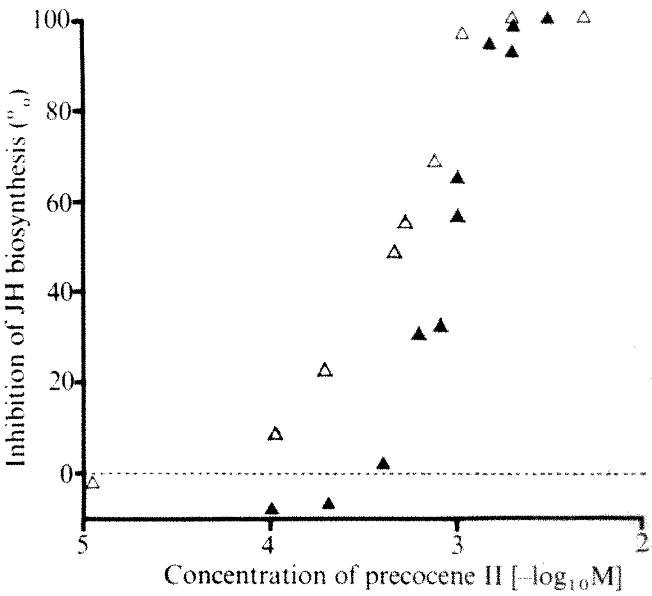

Fig. 1 Effect of precocene II concentration on its ability to inhibit biosynthesis of C_{16} JH *in vitro* during a 3-h incubation with label after a 30-min preincubation. △, Response of corpora allata synthesising spontaneously at their maximum physiological rate; ▲, response of glands taken at a time of minimum spontaneous activity but stimulated with optimum concentration of exogenous farnesenic acid. Each point represents the performance of four, six or eight pairs of glands. The two glands from each member of the group were bilaterally randomised between control and experimental tubes, and preincubated in 0.1 ml of sterile augmented medium TC-199 for 30 min with or without precocene II, at 30 °C; the medium was removed and replaced with the same medium fortified with methyl-^{14}C-methionine to final specific radioactivities of 21–23 mCi mmol^{-1} in different experiments. After 3 h incubation with labelled methionine the entire contents of the tubes were brought to *p*H 4.5 with an equal volume of 1% disodium ethylene-diamine-tetracetate, and analysed for the methyl farnesoate and C_{16}JH that had been biosynthesised[6,9]. Appropriate sections of the chromatograms were counted in 11 ml of 7.3% naphthalene, 0.7% butyl-PBD, 0.04% glacial acetic acid, 11% water, in 1:4 dioxan, after vigorous shaking for 1 h and being left overnight in the dark in a Wallac 8100 LSC set for double-label ^{3}H,^{14}C counting.

the hormone is made from exogenous acid, so that the assay approximates closely a test for the ability of precocene II to inhibit esterification and epoxidation reactions at the end of the biosynthetic pathway. Figure 1 shows that we obtained a very similar result for the inhibition of both spontaneous and farnesenic-acid-dependent synthesis; moreover, the rate of esterification of the ^{3}H-farnesenic acid (that is the rate of formation of both methyl farnesoate and C_{16}JH) showed the same sensitivity to precocene II inhibition as did the terminal epoxidation to the hormone. One interpretation of these results is that the effect of precocene II is manifested most importantly at the penultimate esterification stage in the biosynthetic pathway. The results shown in Fig. 1 also imply that the glands are slightly less sensitive to precocene II when assayed in the presence of optimum farnesenic acid concentrations, than when assayed at the lower rates of flux through the last two stages which obtain in conditions of maximum spontaneous activity in the absence of added precursor acid. A better understanding of this apparent protecting effect of exogenous farnesenic acid might afford a clue to the molecular mode of action of precocene II.

To see whether precocene II has a progressive effect on JH biosynthesis, we examined the effect of varying the duration of the non-radioactive preincubation on the ability of critical concentrations of precocene II to inhibit hormone radiobiosynthesis. We maintained sterile procedures throughout the experiment, and reduced the duration of the labelled incubation to 2 h. Such a reduction in labelling time results in an underestimation of the actual rates of biosynthesis, because of the time-lag in the attainment of full isotopic equilibrium in the newly synthesised hormone from methyl-^{14}C-methionine[6]. The results presented in Table 1 suggest that there is an approximate doubling in the effectiveness of precocene II over the 6 h of our study. This simple interpretation however, assumes that precocene II has no effect on the radiochemical lag period. If precocene progressively inhibits either the uptake or metabolism of methionine so as to increase the time taken for the methyl-donor substrate pool serving the *O*-methyl transferase to reach isotopic equilibrium, this would manifest itself as an apparent enhancement of the inhibition of C_{16}JH biosynthesis. Direct measurements have not been reported of intracellular pools of methionine or *S*-adenosyl methionine[11] in the corpora allata of any species. On the other hand, our results make it unlikely that the glands rapidly inactivate or adapt to the inhibitor.

Although precocene II inhibits C_{16}JH biosynthesis in the corpora allata of adult female *P. americana* only at relatively high concentrations compared with some other compounds tested[4], its action on the corpus allatum is interesting for two reasons. First, it is a natural product, and second it has dramatic 'anti-juvenile hormone' effects in several insects, notably hemipterans[1]. Unfortunately, the chemical identity of the natural juvenile hormone of sensitive hemipterans has not yet been established. Precocene II has only weak activity in adult female *P. americana* as an 'anti-juvenile hormone', which is in accord with the high ID$_{50}$ *in vitro* reported here, and could also be a result of high rates of metabolism in this species. Although we have found that precocene II is an effective inhibitor of C_{16}JH biosynthesis in isolated corpora allata, this does not preclude the possibility that it also interferes with other parts of the juvenile hormone control pathway, such as haemolymph transport and target site recognition, which have yet to be directly investigated. It also remains to be discovered whether precocenes are specific inhibitors of the penultimate step in JH biosynthesis, or alternatively inhibit biosynthesis through nonspecific or indirect actions on cellular metabolism. We do not exclude the possibility that precocenes are general metabolic depressants, or inhibit the synthesis of enzymes in the JH biosynthetic pathway which have a rapid turnover.

Table 1 Influence of preincubation time on percentage inhibition of JH biosynthesis by precocene II *in vitro*

Preincubation time (h)	Precocene II 0.25 mM (%)	concentration 0.5 mM (%)
0	20*	20
0.5	20	52
2	20	60
4	71	91
6	64	98

Groups of spontaneously active corpora allata, bilaterally randomised into control and experimental sets, were preincubated in standard culture medium with or without precocene II for up to 6 h. Incubation medium was aspirated off and replaced with the same medium plus labelled methionine. After 2 h further incubation, the rates of production of C_{16}JH were determined by addition of nonradioactive carrier, extraction, thin-layer chromatography and liquid scintillation counting. The percentage inhibitions shown reflect the performance of not less than six pairs of corpora allata in each case.

*Observed inhibitions of less than 20% are not significantly different from zero.

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Facultative blood–brain barrier and neuronal adaptation to osmotic stress in a marine osmoconformer

In most vertebrate and higher invertebrate animals cellular functions are critically dependent on the relative stability of the chemical composition of the body fluids, a requirement epitomised by Claude Bernard's dictum: "la fixité du milieu intérieur c'est la condition de la vie libre"[1]. In higher vertebrates[2,3], and in insects[4], but not apparently other invertebrates[5,6], the ionic homeostasis of the neuronal environment is augmented by extremely effective blood–brain barrier systems. The nerve cells of euryhaline osmoconformers, however, are exposed to considerable fluctuation in the osmotic and ionic concentrations of the body fluids[7]. As an example we studied the serpulid worm, *Mercierella enigmatica* (Fauvel), which occurs in waters in which the salinities vary from 1 to 55‰ (refs 8–10) and which can withstand relatively rapid variations in the osmotic concentration of the blood of between 43 and 1,620 mOsm (ref. 11). We report here that in spite of such extreme variations

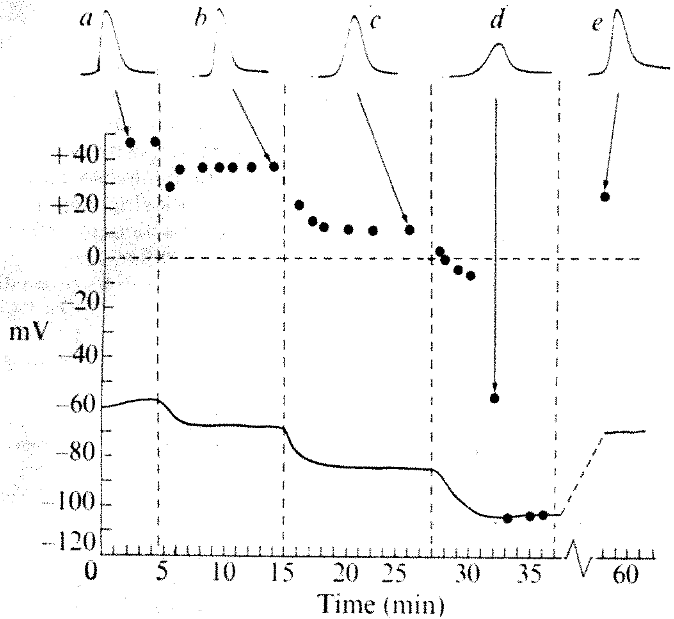


Fig. 1 Effects of isosmotic dilution on action and resting potentials. Isosmicity (1,024 mOsm) was maintained by addition of sucrose to the physiological saline. The intracellular recordings were made with conventional glass microelectrodes (10–20 MΩ tip resistance) from the paired giant axons (30 µm in diameter) in pinned, dissected worms. The axons were stimulated with insulated silver wire electrodes. The standard saline used was based on the ionic and osmotic concentration of the blood of seawater-adapted animals[14] and had the following composition, in mM: Na^+, 482.3; K^+, 30; Mg^{2+}, 77; Ca^{2+}, 31; SO^{2-}_4, 26; Cl^-, 663.8; OH^-, 12.5 and PIPES, 7.5 (*p*H 6.9). *a*, St saline only; *b*, 50% saline in sucrose; *c*, 25% saline in sucrose; *d*, 10% saline in sucrose; *e*, Std saline.

in osmotic and ionic concentration, the axons of *Mercierella* function using ionic mechanisms which seem essentially similar to those of other marine invertebrates, such as the giant axons of the squid[12] or the polychaete, *Myxicola infundibulum*[13]. The resting potential approximates to an ideal potassium electrode (with 58.8 mV alteration for decade change in $[K^+]_o$), the action potentials are sodium-mediated (exhibiting a 55.8 mV alteration for decade change in $[Na^+]_o$) and are blocked by externally applied tetrodotoxin at about 10^{-7} M.

The axonal responses to saline diluted with isosmotic sucrose differed markedly from those observed during exposure to hyposmotic (distilled water-diluted) saline. Isosmotic dilution caused a significant hyperpolarisation, rapid decline in the overshoot of the action potential and eventual conduction block (Fig. 1). The hyperpolarisation probably results from dilution of the external potassium which, at 30 mM, is in a sensitive part of the Nernst slope. The decline in overshoot results from the reduced concentration of sodium ions at the axon surfaces. These responses differ from those observed during hyposmotic dilutions, when axonal function persisted in preparations exposed to dilutions down to 1.0% (Fig. 2) or, in other experiments, to 0.5%. Action potentials also continued in diluted, sodium-free saline (Fig. 2). In contrast, exposure to diluted sodium-free saline caused a rapid decline in overshoot in isosomotic conditions.

Potassium depolarisation of the axon membrane was critically dependent on the osmotic concentration of the bathing medium. Rapid axonal depolarisation occurred in isosmotic saline (Fig. 3). The kinetics of this potassium depolarisation indicate that the inward and outward movements of this cation, between the axon surface and the bathing medium, occurred with half times of 31.0±3.1 and 32.2±3.1 s ($n = 6$), respectively. In hyposmotic saline, however, no appreciable

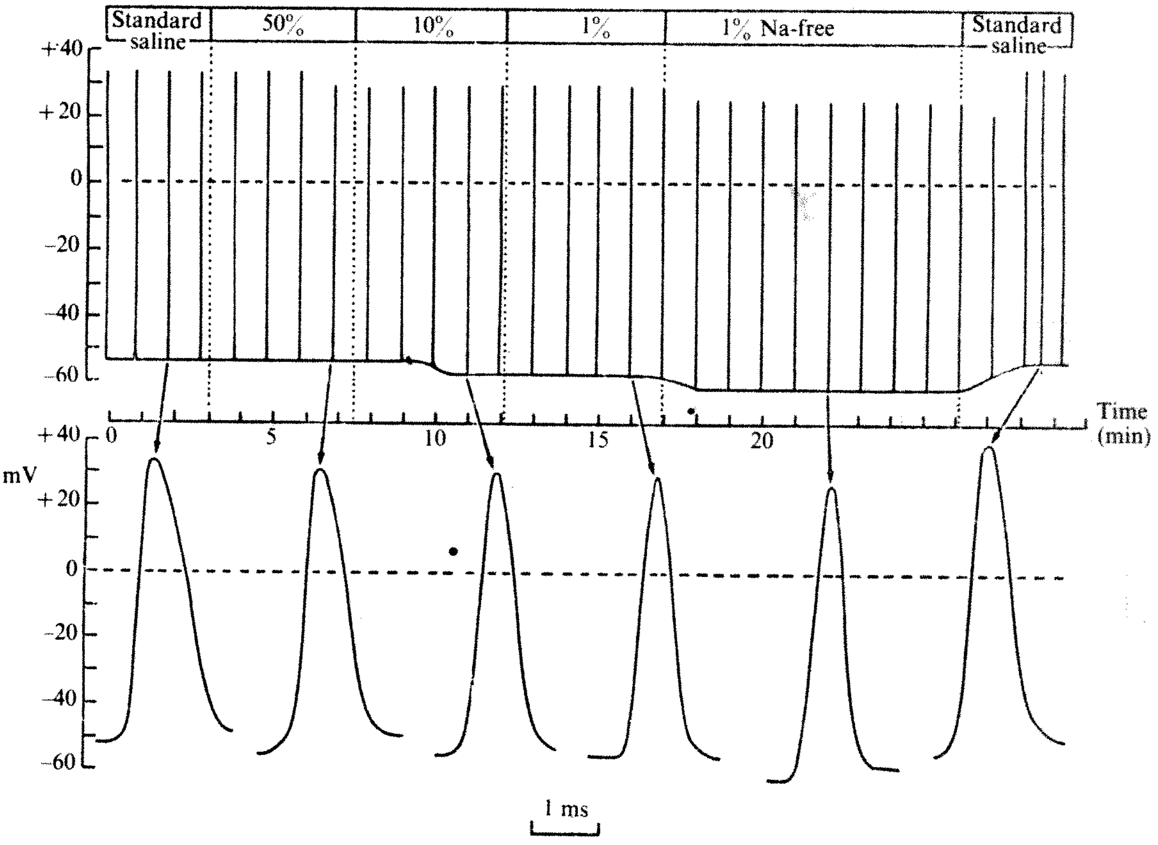


Fig. 2 Effects of hyposmotic dilution on axonal action and resting potentials. Normal physiological saline (1.024 mOsm) was diluted with distilled water down to 1% strength. Sodium-free saline was prepared by substituting Tris ions for Na^+. The potentials were recorded by a transient signal processer and displayed on a chart recorder.

axonal depolarisation was seen after an increase in the external potassium concentration (Fig. 3).

The absence of potassium depolarisation and the lack of effect of sodium-free saline in hyposmotic conditions suggest that a drastic change in accessibility of the axon surfaces occurs during dilution of the bathing medium. This isolation of the immediate fluid environment of the axons was readily reversible, and was abolished when sucrose was added to the bathing medium to restore isosmotic conditions.

Mercierella can survive in distilled water. Axons from individuals adapted to such conditions were able to function in standard saline diluted to 10% strength (Fig. 4*a*). Exposure to sodium-free 10% strength saline caused a rapid loss of axonal function, which indicates that the axon surfaces are accessible in hyposmotically adapted animals. In these conditions further dilution of the bathing medium did not reduce access to the axon surfaces, as exposure to 1% standard strength saline resulted in a rapid decline in the overshoot and irreversible conduction block.

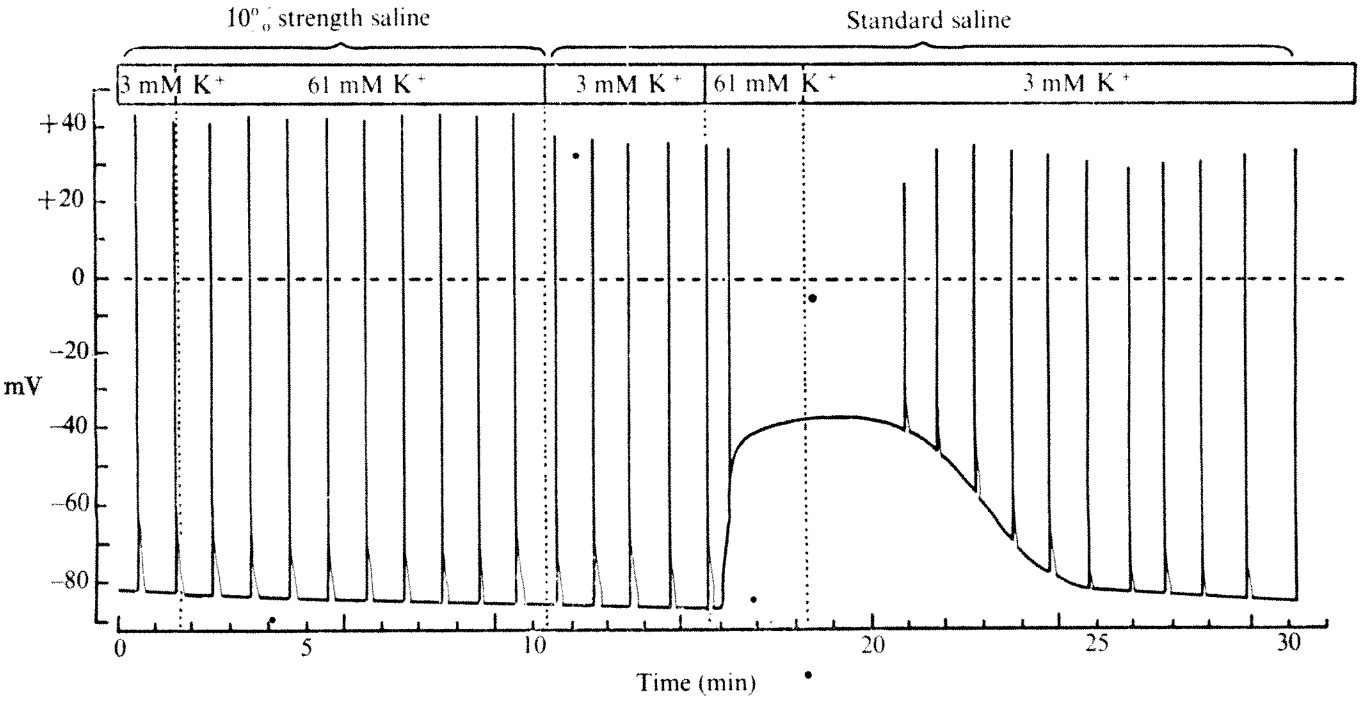
The structural basis of the osmotically-induced diffusion barrier is uncertain. Electron micrographs show that the giant axon is wrapped by narrow glial processes, which are associated with one another by occasional gap junctions, and that this glial–axonal complex is surrounded by extracellular material containing collagen-like fibres. The glial coverage of the axon seems to be incomplete, even in hyposmotic media, so that in these glial-depleted areas the axon abuts directly on to the extracellular material. This organisation is difficult to reconcile with a glial diffusion barrier. Furthermore, the absence of appreciable potassium depolarisation in hyposmotic conditions (Fig. 3) might argue against a cellular barrier, as restricted intercellular access through a peripheral glial layer would be expected to produce relatively large extraneuronal potential changes resulting from depolarisation of the outwardly-directed cell membranes as is the case in insect[15] and crustacean[16] perineuria. Alternatively the osmotically-induced restriction to the access of water-soluble cations might result from changes in the permeability properties of the extracellular matrix which surrounds the axon surfaces.

The accessibility and ability of the axons of hyposmotically-adapted individuals to function at reduced $[Na^+]_o$ (48.2 mM) contrasts with the situation in the axons of seawater-adapted animals. In the latter the axons are protected during hyposmotic stress, and exposure to 10% standard strength sodium (48.2 mM) in isosmotic conditions causes conduction block (Fig. 4*b*). This indicates that the axons can adapt to conduct action potentials at drastically reduced external sodium concentrations.

Adapted axons bathed with 10% strength saline showed smaller overshoots (20.8 ± 1.8 mV, $n = 5$, compared with 38.1 ± 1.1 mV, $n = 20$) and larger resting potentials (69.8 ± 1.5 mV, $n = 5$, compared with 53.6 ± 1.4 mV, $n = 20$) than unadapted axons in normal saline. The total amplitude of the action potential was thus virtually unchanged (Fig. 4*b*). The resting potentials of adapted axons in 10% saline (3.0 mM K^+) were significantly smaller than those of unadapted axons in normal saline containing 3.0 mM K^+ (96.8 ± 2.2 mV, $n = 5$). This suggests that hyposmotic adaptation involves a reduction in $[K^+]_i$ and/or a decrease in potassium permeability of the axon membrane. Similarly, the ability of adapted axons to produce overshooting action potentials at a tenth of the standard sodium concentration (48.2 mM) implies that adaptation involves an appreciable reduction in $[Na^+]_i$. For example, if the sodium selectivity of the active membrane was similar to that of unadapted axons (55.8 mV for decade change in $[Na^+]_o$, then with $[Na^+]_o = 48.2$ mM and an overshoot of 20.8 mV it can be calculated that $[Na^+]_i$ would be approximately 21 mM, compared with 90 mM in unadapted axons bathed in normal saline.

Our observations indicate that two physiological mechanisms are involved in hyposmotic adaptation of this axon. Immediate protection from hyposmotic stress is achieved by a facultative blood–brain barrier which effectively isolates the axon from the diluted body fluids. This isolation enables the axons to adjust more slowly and, eventually, to function when bathed directly

Fig. 3 Effects of increased potassium (61 mM) in hyposmotic (10%) and normal saline. The potassium concentration of normal saline (30mM) was reduced to 3 mM to maintain similar concentrations to that in 10% standard strength saline.



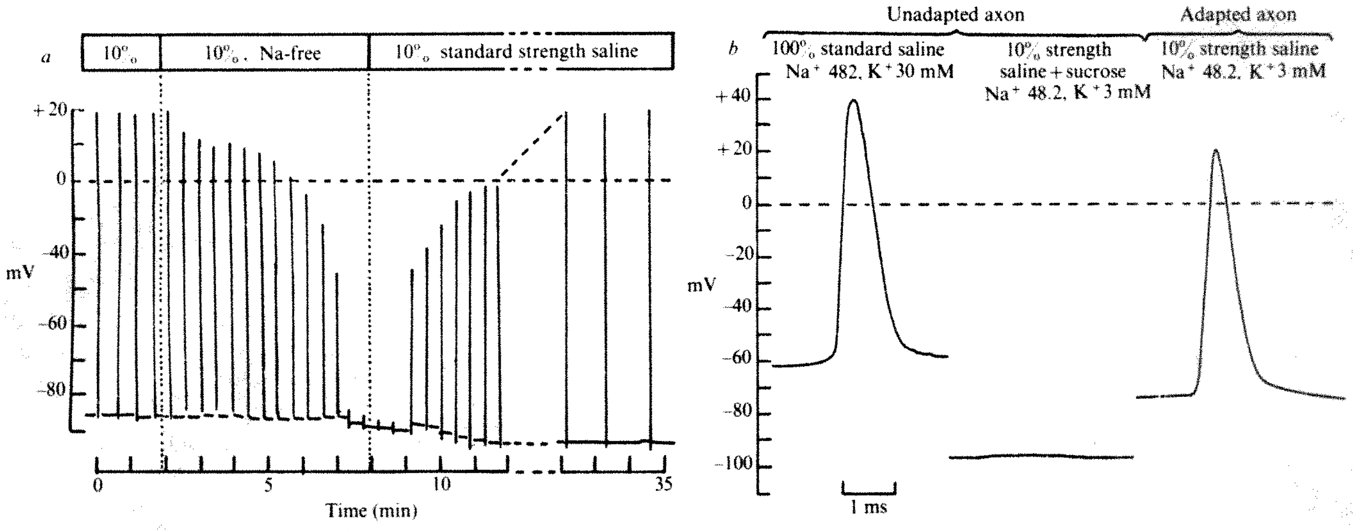


Fig. 4 Axonal action potentials recorded from individuals reared for 10 weeks in flowing distilled water. *a*, The effects of sodium deficiency during exposure to 10% standard strength, hyposmotic, saline. *b*, Action potentials recorded from an unadapted axon, in normal saline, and from a hyposmotically adapted one in 10%, hyposmotic saline. Exposure of the unadapted axon to isosmotic (sucrose-substituted) 10% strength saline caused conduction block and hyperpolarisation of the axon membrane.

by dilute media which would be insufficient to maintain excitability in unadapted nerve cells.

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Isometric contractile properties of single isolated smooth muscle cells

THE use of multicellular strips to study contractile properties of smooth muscle cells rests on the assumption that properties of individual cells are directly reflected in the behaviour of whole tissues. Both the heterogeneity of the contractile state of the cell population in the intact strip, and the complex interconnections between cells and extracellular fibrous elements, however, must influence measured mechanical properties. Methods for isolating viable single smooth muscle fibres from the stomach of the toad, *Bufo marinus*[1,2] now make it possible to make direct measurements of force generation in single cells. I report here a technique for isometrically measuring the force of contraction of a single isolated smooth muscle cell. The method was used to investigate the kinetics and magnitude of force development in a single smooth muscle cell. The results reveal that the maximum force per cm^2 of a single cell is comparable with that of whole tissue. The onset of active force development after stimulation is exceptionally slow. Analysis of this delay suggests that it resides in step(s), perhaps unique to smooth muscle, whereby Ca^{2+} activates the contractile machinery.

Suspensions of isolated single smooth muscle cells were prepared by enzymatic digestion of the stomach muscularis from *Bufo marinus* by a modification[2,3] of the procedure of Bagby *et al.*[1]. Individual cells were tied in a knot at each end around a probe as shown in Fig. 1*a*. One probe was attached to an ultrasensitive force transducer[4] able to measure forces as small as a few μg while the other probe was attached to a micromanipulator. Attachment of a cell to the probes was facilitated by the anionic exchange resin particle at the end of each probe. An electrostatic attraction between the resin particle and the cell facilitates a weak attachment of a probe at each end of the cell. This attachment is strengthened by tying the cell in a knot around each probe using a micromanipulator. The resulting attachment is sufficiently strong to withstand the force of contraction of the cell. When experiments failed they did so quite often because the cell ripped itself in half, rather than pulling free at its attachment. Activation was accomplished by passage of a brief electric current between two Ag–AgCl 'paddles' (350 μm diameter) placed on either side of the cell.

A typical contractile response to a single submaximal shock is shown in Fig. 1*b*. In this cell a peak contractile force of 179 μg was achieved in 11.5 s. In 20 cells stimulated in a similar manner the average time to peak force was 11.0+1.0 (s.e.) s. The mean half-time for relaxation in these cells was 7.7±0.9 (s.e.) s. Peak contractile force depended on stimulus intensity, as shown in Fig. 1*c*. In isotonic studies of similar isolated cells the extent of shortening also varied as a function of stimulus magnitude[2,5]. The maximum contractile force recorded from more than 80 single smooth muscle cells was 2.6 kg cm^{-2}, which is similar to the maximum force measured in intact smooth muscle strips[6,7]. The maximum force per cm^2 produced by a single smooth muscle cell is also comparable with the maximum force produced by a single frog skeletal muscle fibre[8].

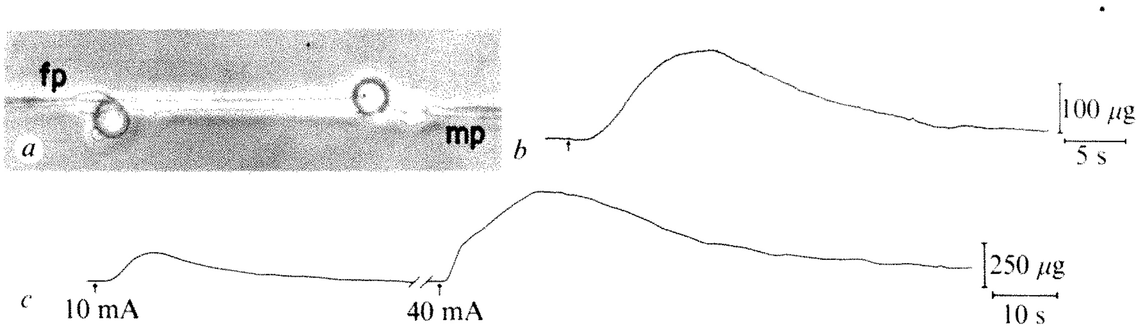

Fig. 1 *a*, Photomicrograph (phase contrast, × 390) of a single smooth muscle cell knotted at each end around a probe to facilitate isometric measurements of force. One probe (fp) was attached to a force transducer whereas the other probe (mp) was attached to a Kopf Hydraulic Microdrive (Model 1207S) or a Burleigh Piezoelectric Translator (Model PZ-30). When using the piezoelectric translator an eddy current sensor (Kaman Sciences, Model KD-2000-.5SU) was used to measure displacement amplitudes and timecourse. The microdrive was used for large amplitude stretches, and the piezoelectric device for small sinusoidal oscillations. Cell length was adjusted so that resting force was between 5 and 12 μg. Experiments were performed at room temperature (19–22 °C). Most cells used were exposed to 10 μM isoproterenol to ensure that they remained relaxed during the knotting procedure. At this concentration isoproterenol has a potent relaxing effect on the isolated cells which can be overcome by electrical or other stimulation[3,6]. *b*, Isometric recording of contractile response of a single smooth muscle cell to a submaximal electrical stimulation. Stimulus was a 1-ms square wave of 14-mA amplitude and occurred at the arrow. Maximum force achieved was 179 μg, or 0.41 kg cm^{-2}. Cross-sectional area of individual fibres on which force measurements were made was calculated assuming the cells to have a circular profile. Cell diameter was measured midway along the length of the cell from video recordings through a microscope; the video image gave a × 500 magnification of the cell. Cross sections of fixed single smooth muscle cells revealed a basically circular profile. *c*, Isometric recordings of contractile response of a single smooth muscle cell to two levels of electrical stimulation. The stimulus, a 1-ms square wave whose amplitude is indicated below the records, was initiated at arrow. Maximum forces achieved were 155 μg (0.55 kg cm^{-2}) and 512 μg (1.81 kg cm^{-2}) in response to 10- and 40-mA pulses, respectively.

Considering the much lower myosin content in smooth muscle (20% of that in skeletal muscle[9]) it is remarkable that similar forces can be generated. Although myosin in smooth muscle seems to be organised into slightly longer filaments (2.2 μm) than in striated muscle (1.5–1.6 μm)[10], the increased filament length *per se* can account only to a small extent (27%) for the apparent increase in force per cm^2 per g myosin. An additional explanation may derive from studies of changes in the ultrastructure[2,11] birefringence[12], and orientation of birefringent 'fibrils' in isolated smooth muscle cells[13] during isotonic contraction, which suggest that contractile elements are oriented obliquely to the cells' long axis. As Rosenblueth pointed out[14], with this arrangement there are more force-generating elements effectively in parallel within the cell than if the same elements ran end-to-end in series, as in skeletal muscle.

There is a considerable delay between electrical activation of a smooth muscle cell and the onset of active force development. The mean latency between a single 1-ms shock of supramaximal intensity and the development of force was 214 ± 13 ms (mean ± s.e.; 60 cells). The time course of the onset of force development in a single cell is shown in Fig. 2*a*, where there is a delay of 170 ms between stimulation and an increase in force. Values for the latency in the 60 cells studied varied from 50 to 500 ms, so that Fig. 2*a* represents one of the shorter latencies. Previously, when preliminary results were presented, even longer latencies were noted, but re-examination of the force recordings containing these very long latencies (>2,000 ms) revealed that the cells were not maximally activated, as judged by the very low values for peak d*P*/d*t*. The latency in the smooth muscle cells reported here is very much longer than that in skeletal muscle in similar conditions (2 ms in frog skeletal muscle at 20 °C (ref. 16)); it is also longer than the minimum latency observed in amphibian cardiac muscle (15–20 ms in frog ventricle[17]). Long delays for development of active force have also been noted in intact strips of smooth muscle. For example, there is a 80–160-ms interval between an action potential and the onset of contraction (measured indirectly) in uterine strips at 30 °C (ref. 18).

Although the rate-limiting step responsible for the long latencies in the isolated smooth muscle cells has not been identified, several potential causes for the long delay can be eliminated. The long latencies cannot be attributed to an effect of isoproterenol (10 μM), present in most experiments because, in the absence of isoproterenol, similarly

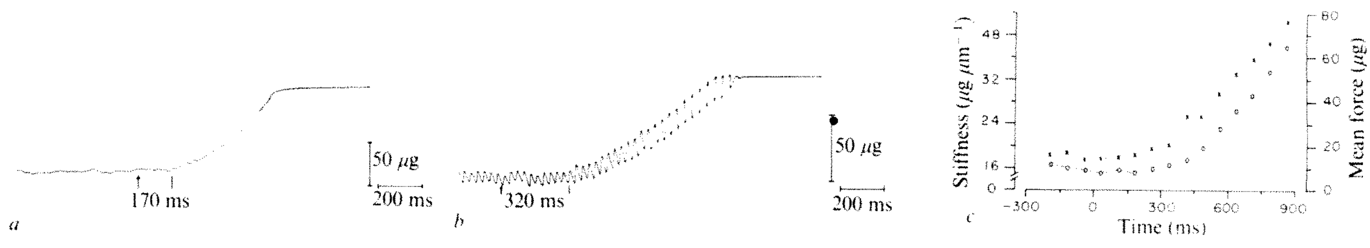

Fig. 2 *a*, Isometric recording of the contractile response of a single smooth muscle cell to supramaximal electrical stimulation. Stimulus (1-ms, 55-mA square wave) was initiated at the arrow on the record. 170 ms elapsed between stimulation and onset of measurable active force. Maximum force developed by this cell was 698 μg (1.1 kg cm^{-2}). Due to the high gain used to facilitate early detection of active force development, amplifier saturates (flat right-hand portion of record) 700 ms after stimulation. *b*, Isometric recording of the contractile response of a single smooth muscle to supramaximal electrical stimulation while undergoing sinusoidal length oscillations. Stimulus (1-ms, 62-mA square wave) was initiated at the arrow. Sinusoidal length oscillations (33 Hz) were 0.5 μm peak to peak. Cell length between knots was 186 μm. Flat portion of the record (right-hand portion of trace) indicates saturation of amplifier at high gain used. Maximum force achieved was 249 μg (0.56 kg cm^{-2}). *c*, Average force (○) and stiffness (×) as a function of time before and after electrical stimulation for the cell in Fig. 2*b*. Stimulation occurred at zero time. Mean force was taken as the midpoint of the force trace in Fig. 2*b*. The plotted values for stiffness represent the mean stiffness during successive 75-ms periods; the latency for the onset of active force development was 320 ms; stiffness did not increase until active force was measurable.

long latencies were noted (254 ± 34 ms; mean ± s.e.; $n=4$). The delay does not seem to be in the ability of the contractile apparatus, once activated, to produce measurable force. If slack within the contractile system, or a delay between attachment of cross-bridges and the initiation of their cycling were responsible for the long latencies, muscle stiffness, which is associated with cross-bridge attachment in striated muscles[19–23], would probably increase before active force development. This is not the case. When muscle stiffness is measured either by applying small sinusoidal length oscillations (10–100 cycles s^{-1}; 0.5–2 μm amplitude) to the muscle or when rapid larger amplitude stretches are performed before and then at various times after stimulation, no increase in stiffness is measured until after active force is produced (Fig. 2*b* and *c*; Table 1). Although it might be argued that stiffness in smooth muscle, unlike that in other muscles, is unrelated to cross-bridge attachment, this is unlikely, because a generally linear relation between stiffness and active force development has been noted in individual fibres (Fig. 2*b* and *c*). A similar relation between stiffness and active force[24] was seen in intact smooth muscle tissues but the unknown contribution of extracellular elastic elements to such measurements makes the interpretation of results on intact tissue uncertain. The possibility that the latency reflects the time required to eliminate slack within the contractile system is further rendered unlikely by the observation that a rapid stretch of 9–11 μm (accomplished as a ramp in 60 ms) given 40 ms after the stimulus did not reduce the mean latency (285 ± 20 ms; $n=7$) below that of a control group (249 + 33 ms; $n=7$). These results indicate that the latency must reside in a step between the stimulus-induced potential change and the attachment of cross-bridges.

Table 1 Relative resistance to quick stretches applied before and after stimulation

Time between onset of stimulus and completion of quick stretch* (ms)	Resistance to stretch† relative to unstimulated cell	Force just before quick stretch‡
50–100	0.96 ± 0.10 (n = 9)	0
100–200	0.94 ± 0.11 (n = 8)	0
1,060	3.21 ± 0.49 (n = 3)	0.12 P_{max}

*Stretches applied as a ramp at 1 μm per 6.2 ms. Stretch amplitude averaged 8 μm; normalised for cell length this corresponds to an average of 5.3% of cell length just before the quick stretch.

†Resistance to stretch was taken as the peak transient increase in force resulting from the sudden length increment. Values are the mean ± s.e. of the ratio of the resistance to identical length increments applied several seconds before and then at the time noted after stimulation. The number of cells is indicated in parentheses.

‡Active force existing in the fibre at the initiation of quick stretch relative to the maximum measured force. Value is the mean for the number of the number of cells used in resistance determination.

Table 2 Effect of a preceding submaximal activation on the latency between supramaximal electrical stimulation and the onset of active force

Conditions	Latency (ms ± s.e.)
Supramaximal stimulus	201 ± 42 (n = 11)
Supramaximal stimulus, applied during submaximal contraction*	166 ± 26 (n = 8)

Latencies were determined after a supramaximal stimulus (1-ms, 50–60-mA square wave) for the number of cells in parentheses. Only latency data from those suspensions where the effect of a supramaximal stimulus alone and the effect of a supramaximal stimulus applied after a submaximal stimulus was tested have been included in the mean latencies.

*Submaximal contraction was initiated by a 10–15-mA stimulus, 1-ms square wave; supramaximal stimulus was applied during the plateau of the submaximal contraction.

On theoretical grounds it is unlikely that the long delay reflects the time required for diffusion of Ca^{2+} from its site of release to the myofibrils. Even if Ca^{2+} had to diffuse from the surface membrane to a myofibril at the central axis of the cell (a 'worst case') diffusional equilibration would be 90% complete within 10 ms (ref. 25). As indicated above, typical latencies are much longer than this. Unlike cardiac muscle[17,26], the latency for force development in smooth muscle does not seem to reflect the time required for a slow but continuous release of Ca^{2+} to achieve a threshold required for observable tension development. Such a release mechanism might be slow in an absolute sense or might be effectively slow due to close matching of rates for Ca^{2+} release and re-uptake. If the latency reflected the time for a slow release of Ca^{2+} to achieve a threshold level, then the latency to a suprathreshold stimulus ought to be greatly reduced in a cell already producing some active force, due levels would presumably be above the hypothetical thresh- to a preceding submaximal activation. In such cells Ca^{2+} hold when the supramaximal stimulus is applied. As Table 2 shows, there is no significant reduction in the latency to a supramaximal stimulus in cells already producing active force due to a shortly preceding submaximal stimulus. A further argument against the long delays reflecting the time required to achieve a threshold level of Ca^{2+} rests on the finding that the latency was not affected either by raising Ca^{2+} in the medium from 1.8 to 20 mM or by eliminating Ca^{2+} from the medium.

These observations on the latency of force generation suggest either that the long delay resides in the coupling of transmembrane potential changes to the opening of a Ca^{2+} gate or that there is a considerable delay between the arrival of Ca^{2+} at the contractile proteins and the formation of cross-bridges between myosin and actin. A possible explanation for the long delays is suggested by a recently reported[27,28] difference in the mode of activation of vertebrate smooth and striated muscle. In smooth muscle it has been proposed[29,30] that Ca^{2+} activation is accomplished by phosphorylation of a myosin light chain, and the delays could be imposed by the phosphorylation. In striated muscles where the latency is much shorter, activation seems merely to involve Ca^{2+} binding to troponin followed by a slight rotation of the troponin–tropomyosin complex[31–35]. Continued studies of the mechanical properties of isolated single smooth muscle cells may aid in further understanding both the activation process and the mechanism of force generation in smooth muscle.

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Increased optical transparency associated with excitation–contraction coupling in voltage-clamped cut skeletal muscle fibres

Contraction of a skeletal muscle fibre is triggered by depolarisation of its transverse (T-) tubules[1], which occurs physiologically with the spread of an action potential into the T-system[2]. The T-tubule depolarisation gives rise to calcium release[3,4] from the adjacent but separate[5,6] sarcoplasmic reticulum (SR). The released calcium binds to troponin, removing the inhibition of actin–myosin interaction and enabling contractile activity[7]. Little is known about the nature of the T-tubule–SR interaction. It has been suggested that movement of charged molecules or dipoles in the T-tubule membrane is the initial voltage-sensitive step in the chain of events leading from T-tubule depolarisation to SR calcium release[8], and membrane charge displacement currents which may reflect such movements have been observed and characterised[8–10]. We report here a study of subsequent changes which may be related to calcium release.

Whole muscles or muscle fibre bundles stimulated to produce propagated action potentials exhibit an increase in optical transparency shortly after the action potential[11,12]. In these conditions the transparency increase follows a time course generally similar to that of latency relaxation[11], the fall in muscle tension which precedes the much larger tension development with contraction[13]. Much of the observed difference in transparency and relaxation time courses may be attributable to distortion of the latter due to asynchronous mechanical activation by a propagated action potential[13]. Latency relaxation has been explained on the basis of a mechanical change in the SR accompanying calcium release[13,14], so the transparency increase may similarly reflect SR changes associated with calcium release. We have investigated the time- and voltage-dependence of the transparency increase using voltage-clamped cut muscle fibre segments prepared so as to retain their contractile ability on electrical depolarisation.

With a high-K, Ca-free relaxing solution (120 mM K glutamate, 2 mM $MgCl_2$, 0.01 mM EGTA, 5 mM Tris-maleate buffer), single semitendinosus or ileofibularis muscle fibres from room temperature-adapted summer frogs (*Rana pipiens*) were cut about 0.5–1 cm from the tendon and mounted in a two pool leucite chamber. A single vaseline gap about 0.5–0.6 mm wide separated the pool containing the closed intact fibre segment from the pool containing the cut end. After mounting the fibre, the pools were changed from relaxing solution to the experimental solutions and experiments run at 3.4–4.6 °C.

Both the voltage difference and total current flowing between the pools were monitored and used with an analogue amplifier compensating circuit to produce a voltage V_m' equal to the fibre membrane potential just beyond the gap in the closed end pool. The compensating circuit corrected for voltage drops along the internal resistance of the length of fibre in the gap in a manner similar to standard 'compensated feedback'[15] and also corrected for leakage current across the gap outside the fibre (our work in preparation). A microelectrode was inserted in the closed fibre segment just beyond the gap in order to set the compensating circuit, but the electrode could be removed after setting the circuit. Thereafter, a voltage clamp circuit was used to maintain V_m' at a holding potential of −100 mV and to produce voltage pulses from the holding potential. Using cut fibres isolated from cold-adapted frogs, strength–duration curves for just detectable movement on depolarisation at 3–4 °C (ref. 16) closely resembled those reported for intact fibres under similar conditions[17]. Cut fibres from room temperature-adapted frogs required larger depolarisations for contraction (our unpublished work).

A reduced size image of a uniformly illuminated slit was focused on the fibre and the slit width adjusted so that the slit image, which was in all cases parallel to the fibre and 170 μm long, occupied the middle third to half of the fibre width. After positioning the fibre and focusing and adjusting the slit under the light microscope, a prism in the microscope was moved out of the light path. This focused the image of fibre and slit on a photodiode used for monitoring the transmitted light intensity. Measured changes ΔI in transmitted light intensity were divided by the light intensity I transmitted by the resting fibre.

In all experiments care was taken to keep the depolarisations of a given duration below the level which produced microscopically visible movement. $\Delta I/I$ records for larger pulses which did produce movement exhibited a second component, a decrease in transparency, which occurred during the falling phase of the transparency increases described below. This component was not characterised.

Figure 1*A* presents $\Delta I/I$ records for pulses of four different durations, with the amplitude of each pulse preselected on the basis of being within a few mV of the threshold for visible movement at that duration. The peak value of $\Delta I/I$ in each case was approximately the same. The $\Delta I/I$ time courses, however, differed considerably. Increasing the size of the depolarisation decreased the ΔI latency and increased the maximum rate of ΔI increase following the latent period. A repeat run using the first pulse (record 1*Ae*) shows that drift in the preparation with time was negligible. The time courses of all records in Fig. 1*A* are too slow for the transparency increase to be originating directly from membrane potential changes across surface[18] or T-tubule[19] membranes. It probably originates from either an SR change accompanying calcium release or from a subsequent contractile filament change preceding mechanical activity. Considering that different amplitude and duration pulses which were all close to threshold for mechanical activity were also all accompanied by about the same peak increase in transparency, the $\Delta I/I$ amplitude seems to be related to either tne amount of calcium released from the SR or to the degree of change in the myofilaments.

Figure 1*B* shows the relationship between V_m' during the pulse and the latency $L_{\Delta I}$ and maximum rate of rise $\mathrm{d}(\Delta I/I)/\mathrm{d}t_{\max}$ of

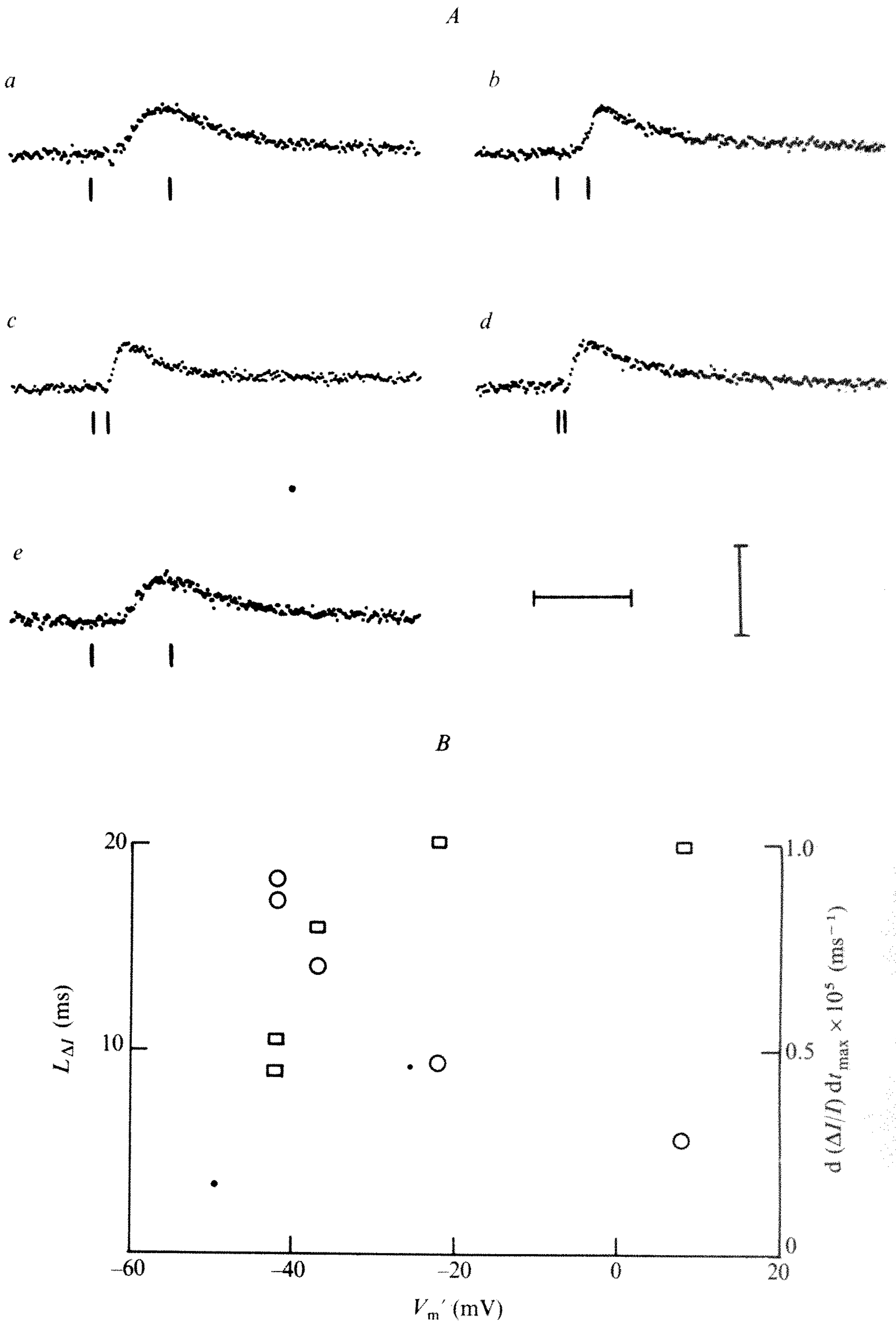

Fig. 1 Transparency changes due to pulses close to threshold for initiating microscopically visible movement. *A*, $\Delta I/I$ records for pulses to the following V_m' values (mV) for the durations (ms) indicated in parentheses. *a*, −42 (50); *b*, −37 (20); *c*, −22 (10); *d*, +8 (5); *e*, −42 (50). Each record gives the average of 10 individual traces. The horizontal calibration bar represents 60 ms, and the vertical 2×10^{-4}. Here and in Figs 2 and 3*A* the vertical lines below each trace mark the first and last interval during the pulse. *B*, Latency ($L_{\Delta I}$) in the onset of the transparency increase (○) and maximum rate of transparency increase $d(\Delta I/I)/dt_{max}$ (□) as a function of membrane potential during the pulse for the traces in part *A*. The parameters were determined from a least squares fit of a straight line through the apparently linearly rising phase of $\Delta I/I$. The closed (tendon) end pool contained 75 mM $(TEA)_2SO_4$, 5 mM Cs_2SO_4, 7.4 mM total $CaSO_4$, 5 mM Tris-maleate buffer, and tetrodotoxin (TTX) at 10^{-7} g ml^{-1}. The open end pool contained 120 mM Cs glutamate, 2 mM $MgCl_2$, 0.5 mM ATP, 1 mM EGTA and 5 mM Tris-maleate buffer (applied 70 min before start of this run). These and all other solutions had *p*H values of 7.0±0.1 at room temperature. Sarcomere length $S = 3.01$ µm, length l_c of intact fibre in the closed end pool = 500 µm, fibre width *d* in the plane perpendicular to the microscope axis = 64 µm. Fibre 68, 3.8 °C. Fibres were illuminated using a battery-powered tungsten-halogen lamp, condensing lenses, a slit of adjustable width, a 525-nm central wave length 25-nm half-peak bandwidth interference filter (Baird Atomic 35-14-7), a microscope objective (10×, 0.25 NA: Nikon 77795) as final condenser, and a glass cover slip as the chamber floor and were observed using a compound microscope with a water immersion objective (40×, 0.75 NA : Zeiss 56 17 02) and 10× wide-field eye pieces (Nikon). A photodiode (Electro-Nuclear PDS-050GB) connected to a current measuring operational amplifier circuit was used to monitor transmitted light intensity *I*. Intensity changes ΔI were monitored by using an a.c. coupled high-gain amplifier (Tektronix 3A9, 1 kHz bandwidth) in series with the photodiode amplifier. A voltage-to-frequency converter (Anadex 1700-5044-00) and digital counters were used to digitise the average output voltage of the 3A9 amplifier for successive 1-ms intervals[9] and a PDP-computer (Digital Equipment Co.) was used to store and signal average the ΔI records. *I* values of resting fibres were monitored by using a d.c. coupled digital voltmeter (Fluke 8000A).

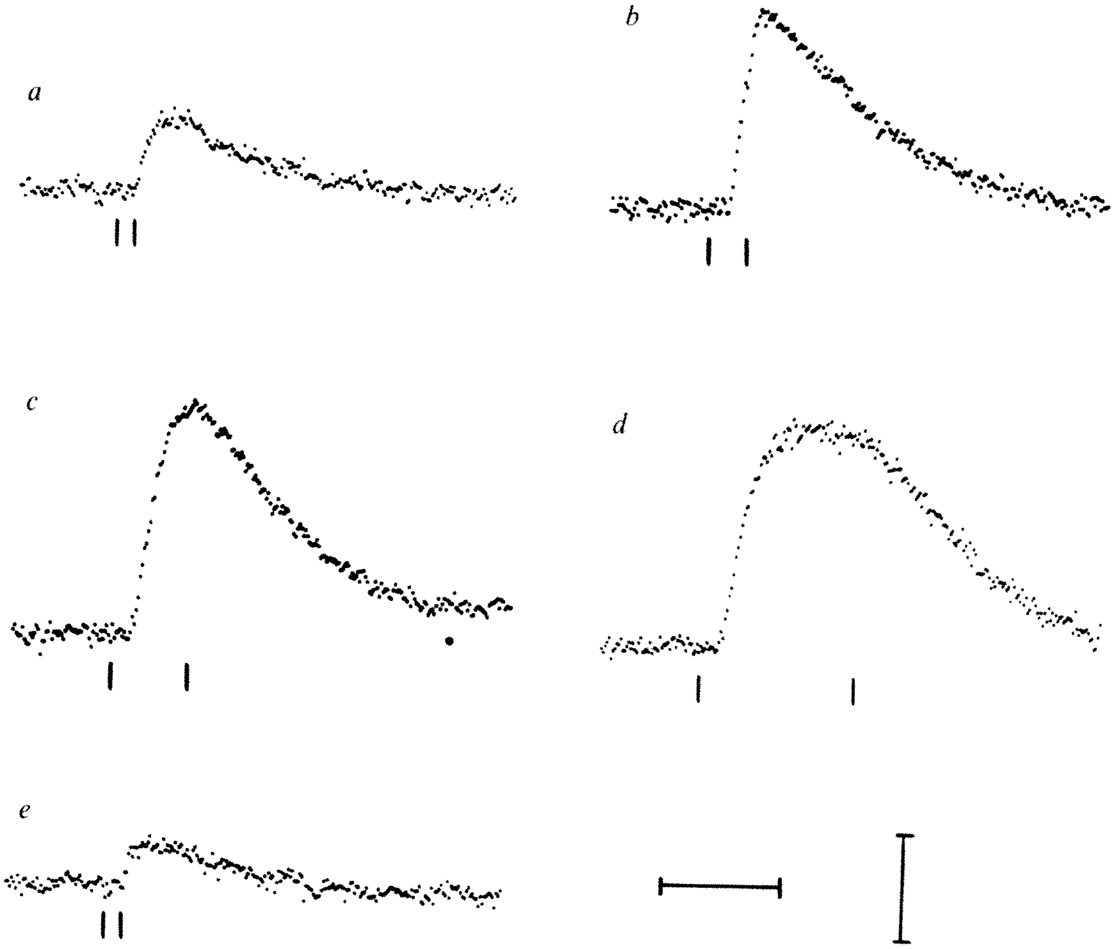

Fig. 2 Effect of pulse duration on the transparency increase. V_m' during all pulses = −14 mV. Pulse durations (ms): *a*, 10; *b*, 20; *c*, 40; *d*, 80; *e*, 10. Each record is an average of five individual traces. Before and after this run, small fibre movements were observed by using the 40- and 80-ms pulses, but not with the 10- and 20-ms pulses. The horizontal calibration bar represents 60 ms, vertical 2×10^{-4}. Solutions used were as in Fig. 1 (applied 200 min before start of this run). S = 2.81 µm. l_c = 510 µm, d = 73 µm, Fibre 69, 4.0 °C.

$\Delta I/I$ for the records in Fig. 1*A*. The minimum latency of 6–10 ms observed in this and other fibres for pulses to V_m' values slightly positive to 0 mV is consistent with the values of 6.5–7.9 ms for the time to onset of latency relaxation in massively stimulated frog sartorius muscles at 0 °C (ref. 20). Latencies such as those in Fig. 1*B* cannot be accounted for on the basis of delays in membrane charge movement[8-10]. If the transparency increase originates from the SR, the latencies suggest that there must be either one or more intermediate steps between charge movement and the SR change or that a number of charges must move before the SR change can occur. If it originates from myofilament changes, the latencies would be made up of both the response time of the SR and the time for released calcium to reach and react with the myofilaments.

In many cases the final decay of $\Delta I/I$ was exponential, with time constants of 33–85 ms in 6 fibres. With other records, a better fit was obtained using an exponential decay to a new baseline level, perhaps indicating the presence of a small amount of another ΔI component in these records.

Figure 2 presents records from another fibre for a run in which pulses of the same amplitude but different durations were used. Increasing the pulse duration from 10 to 20 ms caused a large increase in the maximum $\Delta I/I$ (records *a* and *b*), whereas further lengthening the pulse to 40 ms (*c*) caused only a slight further increase in $\Delta I/I$. Increasing the pulse duration from 40 to 80 ms (*c* and *d*) caused no further increase in amplitude but introduced a plateau phase into the $\Delta I/I$ record.

A third type of experiment involved pulses of constant duration but varying amplitude. Figure 3*A* presents records from this type of experiment carried out using 60-ms pulses with a fibre in Ringer solution which showed no movement for relatively large and long pulses. In this case the signal-to-noise ratio of the $\Delta I/I$ records was sufficiently large that signal averaging was unnecessary and single sweeps could be used. If present, any changes in I for negative pulses to V_m' values of up to −166 mV (record *a*) or for positive pulses to V_m' values down to −44 mV (record *b*) were obscured by baseline noise. Larger depolarisations caused transparency changes which increased with the size of the depolarisation. The variation of peak $\Delta I/I$ with pulse V_m' for the records in Fig. 3*A* is shown in Fig. 3*B*. These peak values represent the maximum $\Delta I/I$ attainable at the given V_m' in this fibre since in each case the pulse ended after the peak in $\Delta I/I$ had been attained. Pulses to voltages slightly positive to 0 mV seemed to have produced a maximal increase in transparency.

The transparency increase which we have studied may originate from either SR or myofilament changes preceding and related to mechanical activity. It does not seem to be a movement artefact, however, because pulses causing no detectable movement can produce transparency increases, and pulses which cause movement also produce a later component of decreased transparency which coincides in time with the movement (our unpublished observation). If the transparency increase is due to myofilament changes, the similarity of its time course to that of latency relaxation would support the hypothesis that latency relaxation also originates in the myofilaments[21] and not in the SR. Independently of the origin of the ΔI signals, the manner in which $d(\Delta I/I)/dt_{max}$ and peak $\Delta I/I$ increase and $L_{\Delta I}$ decreases with increasing depolarisation seems generally consistent with the voltage dependence of membrane charge movement.

The transparency increase time courses reported here resemble the time courses of fluorescence changes seen in intact

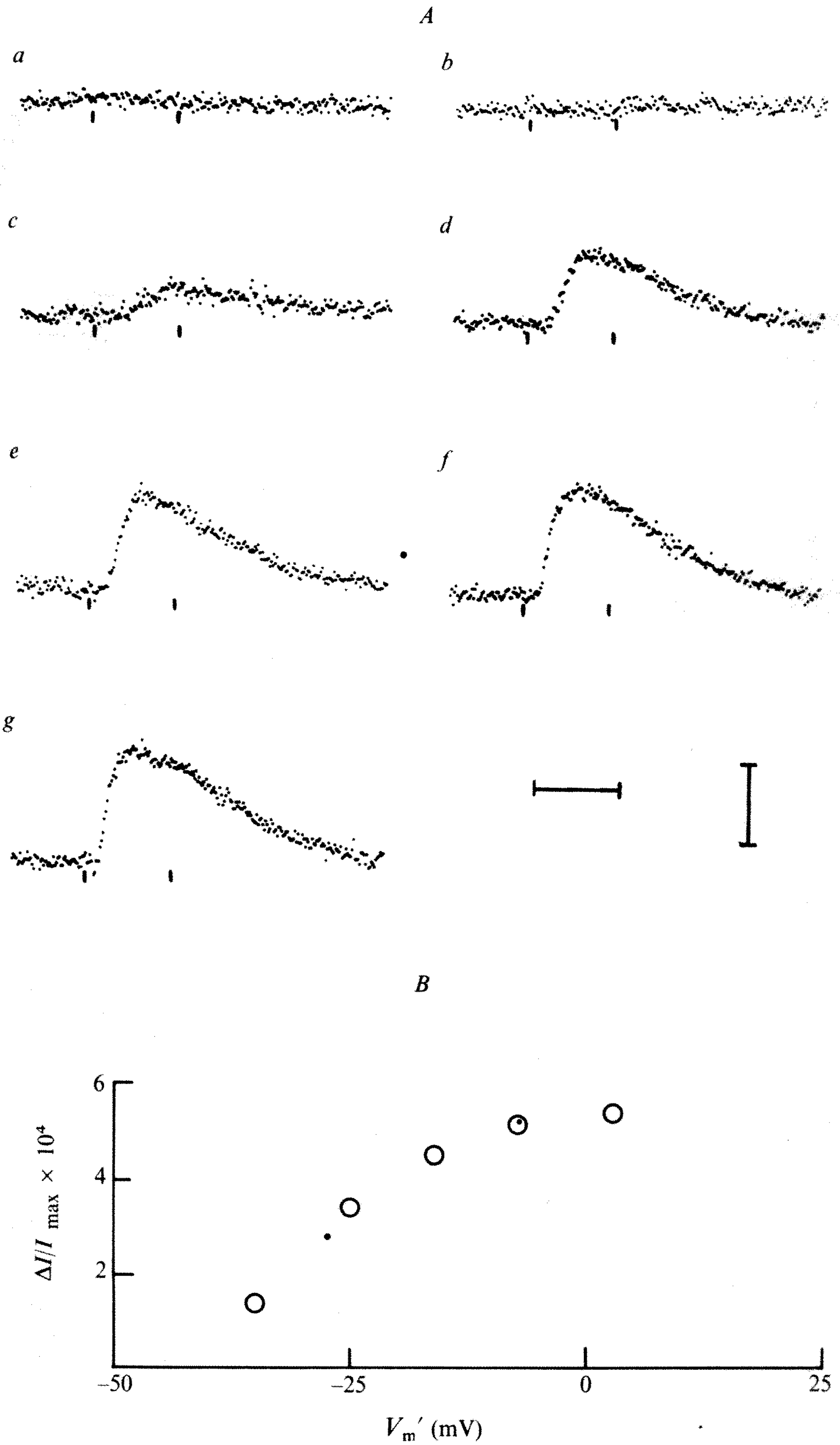

Fig. 3 Effect of pulse amplitude on the transparency increase. *A*, $\Delta I/I$ records for 60-ms pulses to the following V_m' values (mV): *a*, −165; *b*, −44; *c*, −35 *d*, −25; *e*, −16; *f*, −7; *g*, +3. Each record represents a single trace, without signal averaging. The horizontal calibration bar represents 60 ms, vertical 4×10^{-4}. *B*, Maximum $\Delta I/I$ as a function of membrane potential during the pulse for the records in (*A*). The closed end pool contained Ringer's solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1 mM Tris-maleate buffer) with TTX at 10^{-7} g ml^{-1}. The open end solution was identical to that in Figs 1 and 2 except that K glutamate (120 mM) was used in place of Cs glutamate (applied 65 min before the start of this run). $S = 2.69$ µm, $l_c = 500$ µm, $d = 84$ µm. Fibre 64, 3.9 °C.

muscles stained with a penetrating membrane potential-sensitive fluorescent dye and depolarised by current steps applied through fluid electrodes[22], although exact comparison is not possible because of differences in experimental conditions. Since intact single muscle fibres exposed to fluorescent dyes exhibit fluorescence and birefringence changes having similar time courses in response to action potentials[23], it seems likely that the fluorescence changes observed using penetrating dyes[22,23], the second component birefringence change[24] and the transparency increase reported here may all monitor the same events.

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Transfer of virulence *in vivo* and *in vitro* in *Agrobacterium*

TRANSFER of virulence from a pathogenic to a non-pathogenic strain of *Agrobacterium* has been reported to occur *in vivo*[1,2] and is due to the transfer of a plasmid which codes for virulence[3,4]. In the experimental conditions described, virulence transfer can be detected only after several weeks. The reason for this delay is not clear. The most likely explanations are (1) that infrequent transfer occurs long before it can be detected, detection being dependent on a subsequent increase in the ratio of converted to unconverted recipients or (2) that conditions suitable for virulence transfer occur only after donor and recipient have been in contact for several weeks. The main difficulty in distinguishing between these two alternatives is technical. When pathogenicity testing is used to detect virulence transfer, low frequency transfer is impossible to detect unless thousands of inoculations are made at each assessment. It is known, however, that the virulence plasmid codes for characters other than virulence[5–7]. One of these is utilisation of octopine and, as Kerr[8] has pointed out, this could be used instead of pathogenicity testing to detect plasmid transfer. This communication describes both *in vivo* and *in vitro* transfer of virulence using octopine utilisation to detect plasmid transfer.

An experiment was designed to try to detect low frequency transfer of virulence *in vivo*. Strain NCPPB 1001 was used as a donor; it is pathogenic and utilises octopine but is sensitive to rifampicin (25 μg ml^{-1}) and to streptomycin (500 μg ml^{-1}). Double mutation to antibiotic resistance occurs at a frequency of less than 2×10^{-10}. Strain K57 *rif–r str–r* was used as a recipient; it is non-pathogenic, unable to utilise octopine but is resistant to rifampicin and streptomycin. Suspensions of donor and recipient from 48-h cultures on yeast mannitol agar were prepared and mixed in the ratio of 10:1. There were 6×10^8 donor cells per ml. Separate suspensions of donor and recipient were also prepared. These three suspensions were used separately to inoculate tomato seedlings using 15 replicates per treatment. Each week for 5 weeks, three plants from each treatment were removed, the inoculated tissue cut out and macerated separately in 10 ml of sterile distilled water. The macerate (0.1 ml), both undiluted and diluted 10^{-3}, was transferred to agar plates and spread with an L-shaped glass rod. The basal medium contained, per l of water: K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgSO_4.7H_2O$, 0.2 g; $CaCl_2$, 10 mg; $FeSO_4$, 5 mg; $MnCl_2$, 2 mg; and agar (separately sterilised), 20 g. Separately sterilised octopine, 2 g; streptomycin, 0.5 g; actidione, 0.25 g and rifampicin, 25 mg per l were added just before the plates were poured. Actidione was added to prevent fungal contamination. Plasmid transconjugants which combine octopine utilisation of the donor and antibiotic resistance of the recipient grow well on this medium and can easily be distinguished from the 'ghost' colonies of the unconverted recipient. The donor strain is eliminated because it is sensitive to the antibiotics streptomycin and rifampicin. No transconjugant was detected (frequency $<10^{-5}$) 1 or 2 weeks after inoculation. Transconjugants were detected after 3, 4 and 5 weeks at a frequency per recipient of 4.0×10^{-4}, 4.8×10^{-3} and 3.7×10^{-2} respectively. The increase in the frequency of transconjugants with time is significant ($P = 0.011$ by the Kruskal Wallis one-way analysis of variance on ranks).

These results support the hypothesis that infrequent plasmid transfer occurs followed by an increase in the ratio of converted to unconverted recipients. This might be explained by the presence of octopine in galls induced by strain NCPPB 1001 (ref. 7). Converted recipients can utilise octopine and this might give them a biological advantage over unconverted recipients and explain the increase with time in the ratio of these two forms. If correct, perhaps transfer of virulence *in vitro* could be detected by exposing a mixture of donor and recipient to octopine.

We attempted to transfer virulence *in vitro*. Suspensions of donor and recipient were prepared as in the previous experiment. The bacteria in suspension were then deposited on Millipore filters (pore size 0.22 μm) which were then placed on the surface of the basal medium to which octopine (2 g l^{-1}) and actidione (0.25 g l^{-1}) had been added. Two filters from each treatment (donor alone, recipient alone and a mixture of donor and recipient at a ratio of 10:1) were transferred separately after 1, 2, 4 and 7 d to 10 ml of sterile water, the bacteria suspended and plated on the selective medium containing antibiotics. The donor was eliminated: the recipient by itself gave only 'ghost' colonies. The mixture of donor and recipient yielded transconjugants at each assessment, their frequency being 1.4×10^{-2}, 1.9×10^{-2}, 7.1×10^{-2}, and 2.5×10^{-1} after 1, 2, 4 and 7 d, respectively. Again there was a significant increase in transconjugant frequency with time ($P < 0.025$).

If transconjugants are detected because they are favoured by the presence of octopine, removal of octopine should drastically reduce their frequency. An experiment was designed to test this. Bacteria were deposited on filters, as in the previous experiment, but the filters were placed on three different media: (1) basal medium plus octopine plus actidione, (2) basal medium plus actidione and (3) basal medium plus arginine (2 g l^{-1}) plus actidione. Both donor and recipient can utilise arginine as a source of both carbon and nitrogen, whereas octopine (N^2–(D–1–carboxyethyl)–L–arginine) can be utilised as growth substrate by the donor only. The filters were removed after 3 and 7 d, the bacteria suspended in water and plated on the selective medium. Transconjugants were detected only when donor and recipient were exposed to octopine, their frequency being

2.2×10^{-3} after 3 d and 2.5×10^{-1} after 7 d. When octopine was omitted or replaced with arginine, frequency of transconjugants was less than 5.0×10^{-8} after 3 d and less than 1.5×10^{-8} after 7 d. These results further support the hypothesis that transconjugants are favoured by the presence of octopine. A possible interpretation is that octopine promotes plasmid transfer.

The octopine-utilising strains A6, B6 and NCPPB 4 were also used as donors with strain K57 *rif-r str-r* as recipient. After 5 d exposure to octopine in the basal medium recombinants were obtained at a mean frequency of 7×10^{-2}. Presumably the plasmid from nopaline-utilising strains could also be transferred and detected *in vitro* if nopaline, instead of octopine, were added to the basal medium. Unfortunately, nopaline is not commercially available and this restricts its use. But mutants of nopaline strains, constitutive for nopaline metabolism, can be obtained by plating a large number of cells on a medium containing octopine as the sole source of carbon and nitrogen[9]; these mutants can utilise octopine as well as nopaline. Three such mutants of strains C-58, K14 and K24 were selected and used as donors with K57 *rif-r str-r* as recipient using the conditions described above for successful transfer of virulence *in vitro*. Transconjugants were obtained at a mean frequency of 2×10^{-4}. This result indicates that the mutation to constitutivity is borne on the plasmid.

There is still a large group of pathogenic agrobacteria which have to be considered. These are biotype 2 pathogens[10]. They can utilise both octopine and nopaline but only nopaline utilisation is coded for by the virulence plasmid[4-7]. It is therefore impossible to select constitutive mutants for nopaline utilisation by plating these strains on octopine. Nopaline metabolism, however, is inducible; after it has been induced, nopaline strains can degrade octopine[9]. This characteristic was used to obtain transfer of virulence *in vitro* using the biotype 2 pathogens TT 133 and K27 as donors and K57 *rif-r str-r* as recipient. The same procedure as above was adopted except that nopaline (10 μg ml^{-1}), extracted from gall tissue[7], was incorporated into both media, in addition to the other constituents. This level of nopaline was sufficient to induce octopine utilisation. Transconjugants at a mean frequency of 4×10^{-6} were obtained. Although this frequency is much lower than that obtained with other donor strains, it is too high to be explained by mutation of wild-type donors to antibiotic resistance. It seems that most pathogenic strains of *Agrobacterium* can be used as donors to transfer the virulence plasmid to an efficient recipient *in vitro*.

In all the experiments described here, presumed plasmid transfer was detected by selecting recombinants which utilised octopine. At every assessment, 20 recombinants and 20 unconverted recipients were subcultured, purified and tested for pathogenicity. All transconjugants were pathogenic; no unconverted recipient was pathogenic.

In vitro transfer of the virulence plasmid of *Agrobacterium* has been achieved recently by other workers who used the fertility plasmid RP4 to promote transfer[5,6], but this and the following communication[11] are the first reports of plasmid transfer *in vitro* using wild-type donors or their spontaneous mutants.

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Ti plasmids of *Agrobacterium* as conjugative plasmids

THE relationship of the plant-oncogenic properties of *Agrobacterium tumefaciens* to the presence in these strains of large plasmids (the Ti plasmids) has now been convincingly demonstrated in several ways: oncogenic strains cured of the Ti plasmid lose their oncogenicity irreversibly[1-3]; transfer of Ti plasmids to non-oncogenic plasmidless strains confers oncogenicity to these strains[3-7]. The first method used to transfer plasmids was based on observations made by Kerr[8,9], who had developed a method for the transfer of oncogenicity before the association of oncogenicity with large plasmids was known. It was later demonstrated that this transfer of oncogenicity was due to the transfer of the Ti plasmid[3,4]. These observations suggested that in certain circumstances the Ti plasmids could behave as conjugative plasmids in promoting bacterial conjugation and plasmid transfer. This paper and its companion[10] describe experiments confirming that suggestion, making use of the fact that the capacity of *Agrobacterium* strains to utilise the unusual amino acids octopine (N^2-(D-1-carboxyethyl)-L-arginine) or nopaline N^2-(1, 3-dicarboxypropyl)-L-arginine) is coded for by Ti plasmid linked genes[3,5-7,11], and indicate that octopine and nopaline may act in inducing plasmid transfer.

Conjugation experiments were performed with donor and recipient bacteria grown on YEB medium in a shaking waterbath at 28 °C for 12 h. The cells were collected and resuspended in λ-buffer. A mixture of about 10^4 donors and 10^8 recipients was filtered on Millipore filters (pore size 0.45 μm, diameter 25 mm) and the filters were incubated for various periods on solid media consisting either of YEB or octopine or nopaline agar. Filters were then removed and the cells were suspended in λ buffer. After dilution in λ buffer the cells were plated on selective agar media. Filters containing donor or recipient bacteria alone were also prepared and treated similarly.

In a first cross strain ACH-5 (ref. 12) was used as a donor: it is oncogenic, contains a single plasmid 60.6 μm long, utilises octopine but not nopaline as its sole N source and induces tumours which contain octopine but not nopaline. As a recipient we used strain C58-C1, *rif-r, ery-r*. This is a heat cured, non-onconegic, plasmidless derivative of the oncogenic nopaline strain C58 (ref. 4). It was made resistant to 100 μg ml^{-1} rifampicin and 150 μg ml^{-1} erythromycin by two separate spontaneous mutations. To eliminate the donor cells and select for octopine-utilising recipients the mixture from the filters was plated on the basal medium supplemented with 0.5% glucose, 100 μg ml^{-1} octopine, 100 μg ml^{-1} rifampicin and 150 μg ml^{-1} erythromycin. On this medium the donor cells alone did not form any colonies, whereas the recipient strain formed only 'ghost' colonies. When the filters with the mixture were incubated for 1, 4 and 7 d on YEB agar, no octopine-utilising recipients were detected, indicating that the frequency of such transconjugants was less than 1×10^{-8} in these circumstances. When the filters containing the same mixture were incubated for the same lengths of time on octopine (2 mg ml^{-1}) agar, octopine-utilising colonies were detected with frequencies per recipient bacterium of 5×10^{-4}, 1.5×10^{-3} and 6.4×10^{-3}, respectively. The results summarised in Table 1 demonstrate that all

Table 1 Properties of donor, recipient and transconjugant

Strain	Resistance to 100 μg ml⁻¹ rifampycin	Resistance to 150 μg ml⁻¹ erythromycin	Sensitivity to virulent phages of typing set GS 1	GS 2	GS 3	GS 6	GS 18	Ability to utilise octo-pine	or nopa-line	Exclusion of phage AP1	Oncogenicity on pea seedlings	Sensitivity to agrocin 84	Contour length of plasmid (μm)	Fingerprint of *Eco*RI digest of plasmid
Donors														
ACH-5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	60.6 ± 0.8	ACH5 type
B6S3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	(a) 59.0 ± 1.2 (b) 53.8 ± 0.9	(a) ACH5 type (b) x type
C58	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	61.8 ± 1.1	C58 type
Recipient														
C58-C1 *rif-r ery-r*	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	no plasmid	
Transconjugants														
C58-C1 (Ti-ACH5)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	60.4 ± 1.2	ACH5 type
C58-C1 (Ti-B6S3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	59.2 ± 1.2	ACH5 type
C58-C1 *oct-u* (B6S3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	no plasmid	
C58-C1 (Ti-C58)	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	61.8 ± 1.1	C58 type

Resistance to antibiotics was scored as growth on YEB medium containing the antibiotics. Sensitivity to the virulent phages of a typing set was scored as lysis or absence of lysis after spotting of about 10^7 phages ml⁻¹ on lawns of the tested strains in soft agar. The ability to utilise octopine or nopaline was scored by testing whether the tested strains could or could not form full colonies on octopine or nopaline agar. Exclusion of phage AP1 was scored as the absence of plaque formation by this phage on lawns of the tested strains. Oncogenicity was tested on sterile pea seedlings[16], Plasmids were isolated as supercoiled circular DNA molecules by ultracentrifugation[14] and their contour lengths were measured by electron microscopy[17]. Agrocin 84 sensitivity was assayed as described previously[18]. Restriction endonuclease digest fingerprints were obtained after digestion of the purified plasmid DNA with the *Eco*RI endonuclease[19] and separation of the fragments on Agarose slab gels[20]. YEB medium: 0.5% Bacto-Beef extract, 0.1% Bacto-Yeast extract, 0.5% peptone, 0.5% sucrose and 0.002 M $MgSO_4$, *p*H adjusted to 7.2. λ buffer: 0.001 M Tris-hydrochloride (*p*H 7.2), 0.01 M $MgSO_4$. Basal medium: 0.05 M K_2HPO_4, 0.05 M KH_2PO_4, 0.05 M $MgSO_4 . 7H_2O$, 2.5 × 10^{-4} M NaCl, 10^{-4} M $CaCl_2$ adjusted to *p*H 7.2 and 1.5% (w/v) agar (Difco). Octopine and nopaline agar: 2 mg ml⁻¹ of sterile octopine or nopaline separately sterilised and added to the basal medium before pouring plates.

the selected transconjugant colonies (C58-C1 (Ti-ACH5)) were indeed recipient cells and had acquired the Ti plasmid from the donor strain.

In a second cross, strain B6S3 (ref. 13) was used as a donor; this strain is oncogenic, contains two different plasmids with respective contour lengths of 59.0 ± 1.2 μm and 53.8 ± 0.9 μm. It utilises octopine but not nopaline as sole N source and tumours induced with this strain contain octopine but not nopaline. As a recipient we again used strain C58-C1 *rif-r, ery-r*. The cross was performed as described above. When the filters with the mixture of donors and recipients were incubated for 1, 4 and 7 d on the octopine (2 mg ml⁻¹) medium, octopine-utilising recipients were detected with frequencies of 2×10^{-6}, 1×10^{-4} and 2×10^{-4} respectively. As can be seen from Table 1 (C58-C1 (Ti-B6S3)) and Fig. 1, these transconjugant strains have acquired only one of the two plasmids present in the B6S3 donor strain. Indeed, the *Eco*RI digest fingerprint of the plasmid DNA in these transconjugant strains is identical to the *Eco*RI fingerprint of the plasmid DNAs from strain ACH-5 and from the transconjugants obtained with ACH-5 as donor. Furthermore, the fingerprint of the plasmid DNA from strain B6S3 contains as well as all the bands found in the plasmid of strain ACH-5 a number of extra bands. These extra bands are supposed to be derived from the second, so called x-plasmid in strain B6S3. Strain B6S3 must therefore harbour two different plasmids, only one of which is necessary to convey oncogenicity and the capacity to catabolise octopine to a recipient *Agrobacterium*. This plasmid has an identical *Eco*RI fingerprint to the single plasmid present in strain ACH-5.

When the filters with the mixture of donors and recipients were incubated for 1 and 7 d on YEB agar, octopine-utilising recipients were detected with frequencies of 2×10^{-4} and 3.8×10^{-6}, respectively. These colonies have been called C58-Cl *oct-u*(B6S3). As can be seen from Table 1, these are recipient strains that can grow on octopine and not on nopaline but they do not contain closed circular plasmid DNA nor can they induce tumours. Various methods[14] were used to look for plasmid DNA, and also for plasmids either smaller or larger than the donor plasmid; none was found. When used as donors in similar crosses with C58-Cl recipients resistant to 200 μg ml⁻¹ streptomycin and 100 μg ml⁻¹ spectinomycin these colonies were unable to transfer the octopine utilisation marker. It

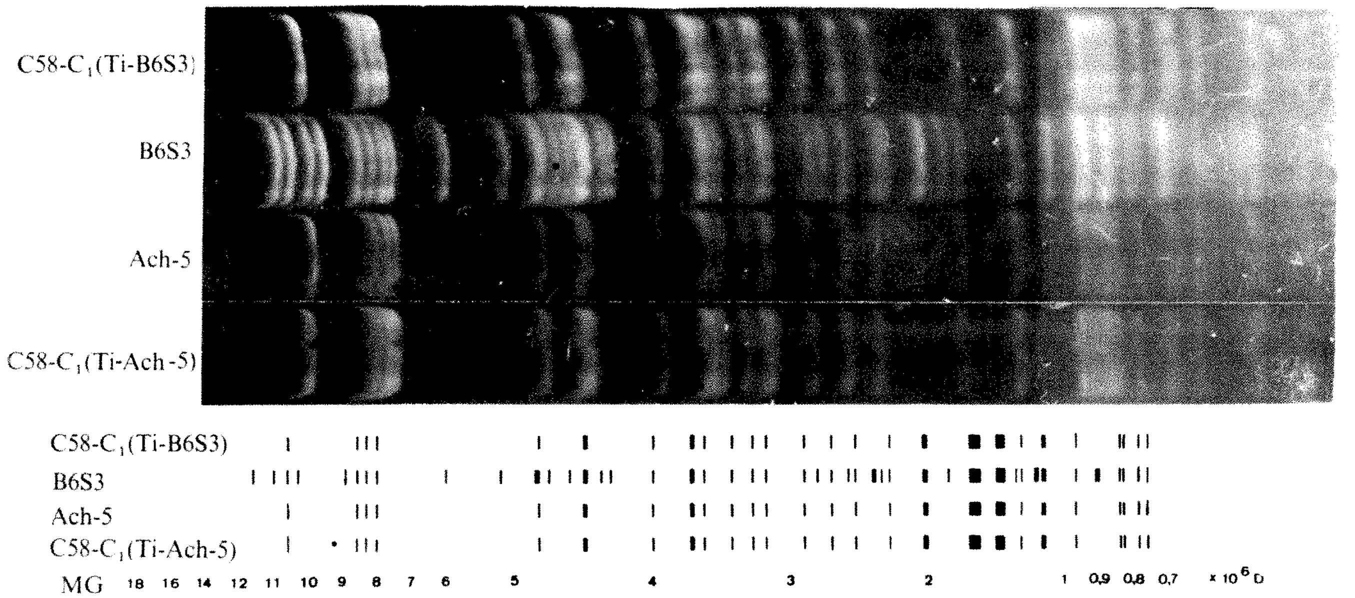

Fig. 1 Fingerprints of *Eco*RI digests of DNA from plasmids of donor and conjugant strains. The *Eco*RI enzyme was prepared according to Greene *et al.*[19]. Plasmid DNA of the indicated *A. tumefaciens* strains was purified on CsCl–ethidium bromide gradients, digested with *Eco*RI endonuclease and subjected to electrophoresis on 0.8% Agarose for 15 h at 2 V cm⁻¹ in the presence of ethidium bromide. The size of each fragment class was determined using *Eco*RI-treated λ DNA as the standard.

seems therefore that these strains have not acquired the whole Ti plasmid from the donor strain. The fact that such non-oncogenic, plasmidless octopine-utilising exconjugants could only be observed with strain B6S3 and not with strain ACH-5 as donor, could indicate that we are dealing here with a phenomenon similar to transduction. Indeed strain B6S3 is known to be lysogenic[13,15], whereas strain ACH-5 is not lysogenic. The main interest of these strains is that they indicate that the Ti plasmid genes that control octopine metabolism could be integrated into the chromosome of *Agrobacterium*.

A third cross was designed to test whether plasmids from nopaline-utilising strains also showed conjugative activity. As a donor strain C58 was used. This strain can degrade nopaline but not octopine and induces tumours that contain nopaline. It harbours a single plasmid 61.8 μm long. As a recipient we used again the C58-Cl *rif-r ery-r* strain. As in the first cross, nopaline-utilising recipients were observed when the filters were incubated on a medium containing nopaline but not when the filters were incubated on YEB agar. The frequency of occurrence of transconjugants was much lower, however, compared with those observed with octopine-utilising donors. Indeed, incubation of the filters on nopaline-containing medium for 1, 4 and 7 d yielded transconjugants with frequencies of 10^{-7}, 10^{-5} and 10^{-4} respectively. As can be seen from Table 1, these transconjugants are oncogenic and have acquired the complete Ti plasmid from the donor strain. The observations described above confirm and extend those described in the companion paper by Kerr *et al.*[10], and demonstrate that the Ti plasmids which determine oncogenicity in *Agrobacterium* strains are conjugative plasmids since transfer of intact Ti plasmids was observed by *in vitro* conjugation experiments. The possibility that the presence of the unusual, crown-gall specific, amino acids octopine and nopaline derepresses the genes involved in this transfer, must be considered seriously and would certainly explain Kerr's observations of *in planta* transfers[8,9].

An alternative explanation would be that the incubation on octopine agar in fact selects for rare exconjugants, increasing their number per recipient. This explanation, however, is unlikely, first, because rare exconjugants were not observed on media lacking octopine or nopaline (level of resolution better than 10^{-8}) and second, the doubling time of octopine-utilising exconjugants incubated on filters on octopine agar is about 3 h. The number of observed exconjugants after 24-h incubation of the filters on octopine agar (see also ref. 10) cannot therefore be explained by enrichment alone. The question therefore arises of whether a mechanism of induced plasmid transfer is involved in the transfer of plasmid-borne genes from the infecting bacteria to the plant cells.

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Tissue enrichments and protein turnover measured with ^{15}N-glycine

VALUES for total body protein turnover (TBPT) measured in man using ^{15}N-glycine[1-3] are comparable with those obtained using ^{14}C-amino acids[4]. Because ^{14}C is radioactive it is of limited clinical use. The stable isotope ^{15}N has a potentially wider application, although its use has been restricted for technical and conceptual reasons[4]. When a tracer labelled with ^{15}N is used to measure TBPT the results are based on the enrichment of urea. This assumes that both urea and newly synthesised protein are derived from the same precursor N pool. Because urea is synthesised in the liver the enrichment of the precursor N pool for protein synthesis in the liver may determine the enrichment of urea. Thus the measurement of TBPT using ^{15}N-glycine may be weighted heavily by the rate of hepatic protein turnover[4,5]. Similar problems may arise when urinary ammonia is the end product (M.H.N.G. and J. C. Waterlow, to be published). The extent of labelling of the precursor N pool is affected by at least two factors. First, after infusion of a ^{14}C-amino acid, its specific radioactivity at plateau is lower in tissues than in plasma[6-8], due to dilution with unlabelled amino acid derived from intracellular protein breakdown. This is termed internal recycling and also applies when ^{15}N-glycine is infused. Second, when ^{15}N-glycine is infused, extensive rapid intracellular transamination occurs especially in liver and kidney, so that the ^{15}N is distributed to other amino acids, which will have a higher enrichment within the cell than in the plasma. Thus, depending on the balance between internal recycling and transamination, the intracellular enrichment of amino acids may be greater or less than the plasma enrichment. To investigate this problem we have infused ^{15}N-glycine, and measured the enrichment of α-amino-N urea-N and

Table 1 Enrichments of N compounds in rat rissue after constant infusion of ^{15}N-glycine

Tissue	α Amino-N	Urea-N (atoms % excess)	Ammonia-N
Whole blood	0.172 ± 0.017	0.118 ± 0.006	0.073 ± 0.011
Liver	0.296 ± 0.019	0.132 ± 0.004	0.134 ± 0.004
Kidney	0.224 ± 0.014	0.136 ± 0.003	0.129 ± 0.004
Muscle	0.170 ± 0.012	0.105 ± 0.006	0.080 ± 0.008
Brain	0.026 ± 0.003	—	0.076 ± 0.002

Values are means ± s.e.m.

Table 2 Total body protein turnover measured using different isotopes and end products

Isotope	Tissue sampled	Total body protein turnover (g kg^{-1} d^{-1})
^{15}N-glycine	Liver urea	36.3 ± 1.9
^{15}N-glycine	Kidney ammonia	36.4 ± 1.7
^{14}C-(U)-tyrosine*	Plasma tyrosine	35.0 ± 1.7 and 39.9 ± 2.6
^{14}C-(U)-tyrosine†	Plasma tyrosine	Between 32.1 ± 2.5 and 41.7 ± 9.6
^{14}C-(U)-lysine‡	Plasma lysine	30.7 ± 1.6

Values are mean ± s.e.m. Protein is assumed to contain 13% nitrogen and 3.12% tyrosine.
*Data from ref. 8.
†Data from ref. 12.
‡Data from ref. 7.

ammonia-N in various tissues. The TBPT calculated from the liver urea-N enrichment gives a value which we consider representative and which agrees with published values based on ^{14}C.

Male albino rats weighing about 390 g were infused through a tail vein with a solution of ^{15}N-glycine (97 atoms % excess, Prochem BOC, London) at a rate of 0.1239 mg ^{15}N per h for 6 h. The rats were then decapitated and whole blood was collected in cold 10% (w/v) trichloroacetic acid (TCA). The liver, kidneys, quadriceps muscle and brain were homogenised in cold TCA. After centrifugation the supernatants were extracted twice with ether and alkalinised with sodium hydroxide in a Leurquin apparatus[10]; the ammonia produced was collected in 0.1 N sulphuric acid by aeration. Urea in the NH_3-free extracts was converted to ammonia by urease (Type III Sigma) at *p*H 5.6 and collected by aeration. Amino-N was extracted from the ammonia-free and urea-free extract by the method of McFadyn[11]. N-enrichment was measured in a double collector mass spectrometer (602 VG Micromass) after reaction of the ammonium sulphate with sodium hypobromite in a vacuum. TBPT was calculated according to the method of Picou and Taylor-Roberts[1]. Significance was tested by the paired *t*-test.

Table 1 shows the enrichment of the three nitrogen compounds in the various tissues. The hepatic α-amino-N enrichment was nearly twice that in whole blood. The enrichment in renal α amino-N was significantly less than that in liver and greater than that in whole blood. This indicates that transamination was extensive in these organs and was a more important determinant of α-amino-N enrichment than internal recycling. The enrichment of α-amino-N was very much lower in brain than in other tissues studied. This is possibly due to a low rate of uptake of glycine by the brain with extensive internal recyclng. Enrichments of α amino-N in muscle and whole blood were the same. There was no significant difference between urea enrichments in liver and kidney. Urea is first synthesised in the liver and then mixes with urea in other tissue pools, so urea samples isolated simultaneously from liver and kidney are originally synthesised at two different times. Since hepatic and renal urea-N have the same enrichment an isotopic steady state must have been achieved between these two pools. The lower enrichment of urea-N in muscle suggests that the muscle pool has not yet reached isotopic equilibrium with the liver pool at 6 h. Unequal enrichment of plasma and erythrocyte urea-N may explain why the enrichment in whole blood was lower than that in the liver and kidney.

There was no significant difference between liver and kidney ammonia-N enrichment or between ammonia-N and urea-N enrichment in these two tissues. Therefore TBPT can be calculated for each rat from the hepatic urea-N and renal ammonia-N data. The results show that there is no significant difference between values for TBPT based on the two excretory products. Furthermore, these results agree closely with those obtained after infusion with ^{14}C-labelled amino acids (Table 2). This substantiates the validity of both methods and suggests that the highly enriched α amino-N in liver, which was not reflected in hepatic urea-N values, does not unduly influence TBPT results calculated from urea-N data.

The total α amino-N of the body does not form a single homogeneous pool. After an infusion of ^{15}N-glycine the ^{15}N label will be distributed to amino acids in a complex way depending on the size of the pools, their varied metabolism and differing turnover rates. Thus it is difficult to interpret or predict the enrichment of total α amino-N in any tissue. Nevertheless, the extremely high enrichment of hepatic α amino-N and the less highly enriched α amino-N in muscle, kidney and blood raises the question of the origin of urea-N. The data obtained show that none of the tissue pools of α amino-N can be identified as the single precursor for urea synthesis. The method of measuring TBPT, however, does not make any assumptions about the precursor enrichment for either urea or protein and does not require this enrichment to be measured. The method requires only that the nitrogen originating from glycine contributes to both protein and urea in the same ratio as the total α amino-N contributes to protein or urea. This assumption is supported by the close agreement between results obtained by ^{14}C and ^{15}N-glycine measurements, and between ^{15}N-glycine and ^{15}N-egg protein measurements[2].

If urinary ammonia-N enrichment is shown to be the same as renal ammonia-N enrichment, then urinary ammonia-N enrichment could be used to estimate TBPT in man. This would shorten the time required to reach plateau considerably and also simplify sample analysis.

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reviews

Functional biology of the mollusca

R. D. Purchon

Living Marine Molluscs. By C. M. Yonge and T. E. Thompson. Pp. 288. (Collins: London, 1976.) £6.00.

BEAUTIFULLY illustrated with sixteen plates (eight in colour) and with 162 informative line diagrams, this inexpensive book fills a major gap in libraries. It will be invaluable to university students; malacologists and palaeontologists professionally concerned with understanding functional morphology and the principles of molluscan evolution; conchologists; and enthusiastic amateur shell collectors.

The text, arranged in eighteen chapters all of a convenient size, is an easily understood narrative reviewing the functional biology and evolution of all the more important molluscan taxa, on a global scale. There is also a wealth of minor detail regarding the species of British marine mollusca.

After a brief introduction, chapter 1 is devoted to an historical review of molluscan studies to the present day. It remains for the reviewer to emphasise that the past half century has seen an explosive growth in the study of the functional biology of molluscs, to which no-one has contributed as extensively as has the senior author of this book.

Chapters 2, 3 and 4 deal with basic features of molluscan organisation and classification, and with the chitons which, with their eight overlapping shell plates and muscular foot, are adapted for movement over irregular hard surfaces. Chapters 5–9 concern the Prosobranchia, dealing successively with "The first gastropods"; "Limpets and top shells"; "Mesogastropods" (which require two chapters); and with "Neogastropods". Here is a wealth of well-explained functional biology and ecological detail. These chapters portray the evolutionary plasticity of the Prosobranchia, and the resourcefulness of the Mesogastropoda in particular.

The next two chapters concern "Opisthobranch sea snails", and "Sea slugs". Reduction of the shell here leads to alternative chemical and biological protection against predators. After lightening, or loss, of the shell several lineages have independently acquired the ability to swim, the better to escape from their enemies. We can appreciate something of the grace and beauty of these delightful animals from the excellent line and stipple drawings here.

Title page of first book devoted entirely to molluscs

By Filippo Buonanni (Rome, 1681)

Five and a half chapters are assigned to the Bivalvia, beginning with an account of the origin of primitive bivalves from a generalised molluscan ancestry, and covering the general organisation and mode of growth of bivalves. In "Evolution and adaptation of bivalves" we see a transition from deposit-feeding protobranchs to suspension-feeding lamellibranchs, with elaboration of ctenidial ciliary sorting mechanisms. These lamellibranchs exploit every possible aquatic habitat and mode of life, as is shown in chapters 14–16: byssal attachment to hard surfaces, with the adoption of heteromyarian or monomyarian form; swimming; shallow or deep burrowing in sediment, with a return to deposit feeding in the Tellinacea; commensalism; and boring in rock and timber by pholads and shipworms.

Chapter 17, "Anomalous bivalves and scaphopods", deals first with the Anomalodesmata, which lack hinge teeth and in some of which there is a lithodesma. The Verticordiidae indicate a transition from filter feeding to a scavenging or carnivorous habit, as also in the related Septibranchia. This was a necessary pre-adaptation towards life in abyssal sediments. Mention is made of the Watering Can Shell, *Penicillus*, which calls for a special effort by functional morphologists, who happen to be well-placed in the Indo–Pacific Region, to elucidate the general biology and growth processes of early stages of this puzzling bivalve.

The Scaphopoda are shown to be either microphagous (*Dentalium conspicuum*), or carnivorous (*D. entalis*).

Chapter 18, "Cuttlefish, squid and octopus", relates the mantle cavity of these actively swimming molluscs to those of other molluscan classes. Attention is given to newly gained knowledge of the biology of the Pearly Nautilus, which moves slowly in moderately deep waters. Transition from ciliary to muscular ventilation of the mantle cavity permitted fast swimming, and required a high metabolic rate. This permitted the exploitation by cephalopods of waters from just above the seabed to the surface of the sea. In such pelagic hunters the statocysts, eyes, and brain are well developed, as also the tactile sense in octopods, which have secondarily returned to the seabed. A brief account is given of learning and memory in the octopus, and how knowledge of this has been gained by simple experiments.

The substance of the book is surveyed in an Epilogue. The mollusca arose with the contrasted advantages accruing from a bilaterally symmetrical body and a radially symmetrical protective shell; these, with the versatile radular apparatus, provided a basis for diversification to suit widely different habitats and modes of life. By acquisition of neutral buoyancy and muscular jet propulsion the cephalopoda outstripped all other invertebrates and now compete on equal terms with the marine (but not freshwater) Teleostei.

Erato voluta (Fig. 34, p86), until recently regarded as a cypraeid, should be assigned to the family Eratoidae together with the genus *Trivia*. *Xylophaga* (p218) is reported as being unable to swallow fragments of the wood through which it bores, but this is not so; it has a moderately large caecum which is normally packed with wood fragments.

There is a useful list of recommended books for further reading, and there is a thorough index. Typographical errors are rare. □

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Chromosome studies in cancer cells

Cytogenetic Aspects of Malignant Transformation. (Experimental Biology and Medicine: Monographs on Interdisciplinary Topics, Vol. 6.) By N. B. Atkin. Pp. iii+168. (S. Karger: Basel, London and New York, 1976.) SFr/DM74.

THIS book is a critical review of the present state of chromosome studies in cancer cells by an author who has worked on these problems for very many years. Essential concepts relating to chromosome changes in malignant transformation are old and represent views which are now 'rediscovered' with more precise tools. In his evaluation of new evidence, the author draws on substantial material of his own, and the book therefore gains a particular authority. Such studies are *central* to the cancer problem, more so than many erudite and sophisticated lines of research, intellectually more favoured by grant-giving authorities. The new evidence clearly bears out that chromosome changes are nonrandom, clustering on particular chromosomes; but many patterns are not yet discernible at histological and biochemical level, although such distinction must be present.

Some expected conclusions are not drawn by the author. The monoclonal nature of neoplasms sets a low limit to the number of cells capable of forming a clone in a tissue, the target cells for transformation. Other general principles seem to emerge. With a primitive stem cell transformation comes tumour-specific numerical chromosome imbalance, with a more derived stem cell transformation, single gene effects, that is, balanced translocations, and low numerical variation.

It may be that transformation is a process by which a clonogenic cell takes a retrogressive step in its phylogenetic pathway, thereby gaining a proliferative advantage over other cells. Further transformation often occurs within the clone. This study of 'embryogenesis in reverse' represents a formidable task, not likely to be quickly solved unless supporting disciplines work with these ideas in mind.

S. Muldal

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Membrane separation

Membrane Separation Processes. Edited by P. Meares. Pp. ix+600. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl. 250; $96.25.

MEMBRANE separation processes, despite a long history, can only recently be said to have reached a stage of development where large scale practical applications are possible. At the same time, as a result of very many basic researches, the physics and chemistry of membrane processes have been clarified and a general formulation in terms of irreversible thermodynamics has been developed. It is therefore timely that a monograph on the theory and practice of membrane science should have appeared, in which actual and potential applications have been particularly emphasised.

The book is a cooperative effort to which well-known specialists in various technically significant areas have contributed. Such texts have both advantages and disadvantages—expertise in every topic covered in some, but some omissions, overlap and unevenness in others. This presentation, under the editorship of Professor Meares, has had considerable success in minimising the disadvantages, and the contributors have ensured a high standard in the areas chosen.

The coverage can best be seen from the following chapter headings: the physical chemistry of transport and separation by membranes; liquid permeation through polymers; ultrafiltration; reverse osmosis in desalination; hollow fibres in reverse osmosis, dialysis and ultrafiltration; electrodialysis; piezodialysis; gas separation by selective permeation; hydrocarbon separation by liquid membrane processes; enzyme membranes; separators and membranes in electrochemical power sources; ion-selective membrane electrodes; treatment of aqueous wastes and foods by membrane processes; and biochemical applications of membranes.

The book is well produced despite some printing errors, such as a mix-up in the middle of p201 and a misspelling of "repetitive" in the diagram on p94 and elsewhere. The contributors and editor are to be congratulated on a useful and comprehensive task well completed. The price of this book has unfortunately been set so high that many potential purchasers may be deterred and some libraries will also need to consider whether their funds permit them to buy it.

R. M. Barrer

R. M. Barrer is Professor in the Department of Chemistry at Imperial College, University of London, UK.

Cyano complexes of transition metals

The Chemistry of Cyano Complexes of the Transition Metals. (Organometallic Chemistry: A Series of Monographs.) By A. G. Sharpe. Pp. xi+402. (Academic: London and New York, 1976.) £10.40; $26.25.

THE publication of a comprehensive and up-to-date review of the cyano complexes of the transition metals is particularly timely. There is currently a rapidly growing interest in a whole new range of the related organic isocyano metal complexes, and it is appropriate that this volume indeed appears as a member of the excellent series of monographs on organometallic chemistry, edited by Maitlis, Stone and West.

Although the study of the metal cyano complexes goes back to the early eighteenth century, they continue to be a source of interest and research. Further, they present some of the most elegant examples for the teaching of the main principles of the structure and theory of the coordination complexes of the transition metals.

An introductory chapter deals with the general aspects of the cyanide ion along with the relevant structural, spectroscopic, thermodynamic and kinetic background. The rest of the book deals essentially with factual knowledge of the cyano complexes by individual groups in the Periodic Table, from the lanthanides to the copper and zinc groups.

The past fifteen years have seen a considerable growth in the literature of these complexes, and the author has surveyed this very extensive area with remarkable thoroughness. He does not, however, hesitate to draw attention to scientific results that he regards as either inconsistent or possibly unreliable.

The book contains over 1,800 references to the chemical and physical literature, but only a very brief subject index. Each chapter is, however, so consistently organised that I found little difficulty in finding what information, if any, was available about any particular cyano metal complex system.

Most organometallic and inorganic chemists will want this book on their shelves, not only as a valuable source of reference, but also as a pointer to many intriguing potential research areas.

E. W. Abel

E. W. Abel is Professor of Chemistry at the University of Exeter, UK.

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Duke University Medical Center

This volume in honor of Kenneth S. Cole expands upon his pioneering research in membrane biophysics. Outstanding contributors present the latest advances in the areas of Cole's most significant discoveries in membrane capacitance and conductance. There is also extensive coverage of new areas of membrane research, including the measurement and analysis of fluctuation phenomena and uses of the voltage clamp in cells other than the squid giant axon. Volume 5 in the FASEB Monographs series. 195 pages, illus., $23.40/£12.30

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Synaptic transmitter substances

Neurotransmitter Amino Acids. By Neil Davidson. Pp. viii+179. (Academic: London and New York, 1976.) £5; $12.75.

In the past ten years there has been a rapidly growing interest in certain amino acids such as GABA, glycine and glutamate which are now generally believed to be important synaptic transmitter substances in the vertebrate central nervous system. Neil Davidson has undertaken the formidable task of writing a short book in which he aims to cover all aspects of neurobiology relevant to these amino acid transmitters.

The three opening chapters, which together occupy just over two-thirds of the book, provide an excellent account of the evidence that glutamate and aspartate, glycine, and GABA, are central transmitters. The references are usually well chosen but it is surprising that there is no mention of *N*-methobicuculline or the immunohistochemical localisation of glutamate decarboxylase (GAD). The latter omision is particularly surprising since the localisation of GAD is potentially a valuable method of identifying GABA-operated synapses.

The author is at his critical best when discussing problems of electrophysiology and particularly the interpretation of experiments in which drugs are applied to neurones by iontophoresis. More neurochemical problems involving subcellular distribution, autoradiography and uptake, however, are not so critically assessed. For example, the author suggests that the presence of a high affinity uptake mechanism is a criteria for an amino acid transmitter, a view against which there is now much evidence.

There is an adequate chapter on taurine but the account of amino acids in retinal neurotransmission is rather disappointing. The role of GABA in the retina is discussed but taurine, glutamate and aspartate are barely mentioned, although there is much inferential evidence that they may be retinal transmitter substances.

The last two chapters provide an excellent account of the possible role of amino acids in presynaptic inhibition and a review of the behavioural and clinical correlations in studies of the neurotransmitter amino acids.

The book has a rather large number of typographical errors but of more consequence is the use of a bewildering number of units. For example, the concentrations of amino acids are expressed in at least five ways making ready comparisons difficult. It would be helpful if in future editions the author would recalculate amino acid levels in one unit such as μmol g^{-1}. These are, however, minor criticisms, and the author is to be congratulated on producing a very readable introduction to amino acid transmitters. This book will be of great value to both undergraduate and postgraduate students, and I suspect that many involved in research on amino acid transmitters will keep a copy of this book readily available.

M. J. Neal

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Gerontological observations

The Ageing of Connective Tissue. By David A. Hall. Pp. vi+204. (Academic: London and New York, 1976.) £4.80; $10.50.

Over the past decade considerable advances have been made in our understanding of the biosynthesis and structure of the components of connective tissue. The deterioration of connective tissue is an obvious fact of ageing, and connective tissue biochemists should be encouraged to apply this new fundamental knowledge and accelerate the emergence of gerontology from its classical descriptive phase. Certainly it is becoming increasingly obvious that any breakthrough in the understanding of ageing can only come from studies at the molecular level. Dr Hall has therefore chosen his time well to write his monograph.

The book is well organised to provide the reader firstly with a brief resumé of the current theories of ageing and then with an overall view of the breadth of involvement of connective tissue components in various tissues, starting at the macroscopic level and working gradually down to the complex molecular level.

The book does, however, contain a number of errors, some obvious and some not so: for example, the gal–glc disaccharide is stated as being attached to hydroxyproline instead of hydroxylysine; the statment that $(\alpha 2)_3$ exists in tissues; the stated presence of hydroxylysinonorleucine in elastin; and a number of incorrect cross-link formulae. There are also a number of surprises: for example, quotation of the old, unconfirmed observation that cellulose is present in older human skin; the electron micrograph picture of metacollagen (thermally denatured skin collagen); the considerable over-emphasis on pseudo-elastin (an unfortunate name, since it is surely no more than highly cross-linked impure collagen). The statement that elastase, a serine protease similar to chymotrypsin, has no effect on collagen is incorrect, a fact which throws some doubts on the conclusions drawn from these experiments.

I did not, therefore, feel that the promise of the first half of the book was fulfilled when the biochemical basis of the age changes was discussed. More importantly the emphasis in this part of the book on old unconfirmed and often confusing work, together with the numerous errors of fact that have been overlooked, mean that the book has to be read with considerable wariness. This is particularly important since the stage is now set—and Dr Hall has played no small part in this—for some critical studies on the role of connective tissue in the ageing process. Despite these reservations, it is hoped that this compact and inexpensive book will engender the right kind of enthusiasm for establishing the biochemical and molecular basis for gerontological observations.

Allen J. Bailey

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Multitude of fishes

Fishes of the World. By J. S. Nelson. Pp. xiii+416. (Wiley-Interscience: New York and London, October 1976.) $31.85; £18.50.

MODERN estimates of fish abundance have reached 30,000 species, although the present author arrives at a conservative and precise 18,818. Nowadays, to encompass the fishes even at family level is a daunting task, further complicated by much revision of their higher classification within the past fifteen years.

The purpose of this book is "to present a modern introductory systematic treatment of all major fish groups", the basic units being all extant, and selected extinct, families of fishes, from jawless ostracoderms to choanate crossopterygians. As a preliminary, protochordates and even conodonts are also discussed. The family category is, of course, a subjective taxon, but the author is not extravagant by previous standards in recognising 450 of these in 46 orders of true fishes. Overall classification follows accepted recent schemes, although more radical alternatives are mentioned. Each family receives a common name, a concise statement of distribution, essential diagnostic features, sometimes further division into subfamilies, and biological notes when of better known status. A line drawing of a typical species is provided for most families. Appendices contain a simplified classification and a series of 45 world distribution maps. A useful bibliography of about 600 references (though none more recent than 1973) and a systematic index, complete the work.

This is a pleasing book, not only in much of its content but also in typography, format and illustration. Factual coverage for each family is necessarily brief, but random consultation proved consistently successful even for such diverse forms as *Schindleria* (the lightest vertebrate), parasitic eels (Simenchelyidae), the dreaded candiru (Trichomycteridae), and Baikal oilfishes (Comephoridae). Treatment of the gobioid fishes—representing perhaps one-tenth of all living teleosts—seems restrained and practicable in comparison with more frenetic and provisional specialist schemes.

Among shortcomings, fossil groups receive uneven and often perfunctory attention, out of keeping with current efforts to integrate classification over the entire time range of fishes. A work for general reference also needs a synopsis of fish anatomy, especially osteology, to explain diagnostic characters, perhaps replacing much of the rather generalised introduction and the 24 pages devoted to maps. Use of recognised terminology for faunal complexes could have given a more precise and biologically significant statement of family distribution. Finally, comparison with a recent Russian work by Lindberg, also entitled in translation *Fishes of the World,* and involving the same publishers, emphasises the absence of a key to families from Nelson's account. Keys are not only altruistic but also cathartic against taxonomic subdivision. In a number of ways, by accident or design, the two monographs are unfortunately complementary rather than competitive and it is a pity that a little more on bones, fossils, and identification could not have been included between the relatively expensive covers of the one now reviewed.

P. J. Miller

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Radioimmunoassay

Radioimmunoassay of Biologically Active Compounds. By C. W. Parker. Pp. xiv+239. (Prentice-Hall: Englewood Cliffs, New Jersey, 1976.)

RADIOIMMUNASSAY is a technique the possibilities for which were realised, at least partly, by endocrinologists a good many years ago. They are, however, far wider than is even now recognised. In principle any soluble substance with which a specific antibody can react is capable of being estimated at the nanogram level or better, even in the presence of large quantities of nonspecific material. At one extreme, therefore, proteins, which are intrinsically immunogenic, and at the other simple chemicals, such as drugs which can be stably attached to 'carrier' proteins and made immunogenic, are open to estimation in grossly heterogeneous mixtures, such as serum and crude tissue extracts. The intermediate group, the medium sized polypeptides of about six to about fifty residues—a group which includes many important hormones—is in fact the trickiest problem. But essentially the problem boils down to good chemistry, so as to attach one's substance undamaged and the right way round; and competent immunology, so as to raise antibodies of—and this is not always so easy—satisfactorily high affinity: which just means that the antigen–antibody complexes must remain bound even at very high dilution. The sensitivity of the test—that is, the smallest concentration per millilitre which can be quantitated—is directly dependent on the affinity attained.

The great strength of this book, which can be recommended without reservation, is that Dr. Parker has realised that the principles and practice of immunogen preparation and of immunisation are in importance equal to or greater than, the details of the actual tests, so that the former are considered at length and with invariable common sense. He treats clearly, thoroughly and from first principles problems of affinity and of cross-reactivity, and of factors in tissue extracts or serum such as degradative enzymes, which may affect assays. He also refers to methods alternative to the standard ones—for example when dealing with the separation of free from antibody-bound antigen, he considers chromatographic or molecular sieve separation as well as the more conventional salt-precipitation or double-antibody techniques.

There are two techniques related to radio-immunoassay which may be of increasing importance in the future, to which some but no very extensive reference is made. These are the use of endogenous blood or cell receptor proteins instead of antibody, and the method known affectionately as Eliza, the enzyme-linked immunoassay. About the first, the general conclusion is that it is usually easier to raise an antibody than to purify a specific receptor protein: I would suggest, however, that in some cases—using, for example, suspensions of cultured cells or blood leukocytes—a highly sensitive assay might be contrived for quite small radiolabelled substances, which might be difficult by any other method. As far as Eliza goes, since this involves an antibody, practically everything in this book is as applicable to it as it is to a radioimmunoassay. The great advantage claimed for Eliza is that it is not dependent on a large and expensive counter which is always breaking down, but on a simple colorimeter. The whole question is whether an enzyme assay ending in a coloured product (generally using peroxidase or alkaline phosphatase) can be made as sensitive as a count of radioactivity. The evidence is, rather surprisingly, that under favourable circumstances it can.

This book is the most intelligent one which I have met for developing and advancing the subject; it cannot fail to be of continuing importance in biology and medicine.

P. G. H. Gell

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nature

February 17, 1977

Choosing the top people

It is taking some time to find a successor to Sir Sam Edwards as chairman of the UK Science Research Council (SRC), the body that oversees the spending of an annual £100 million on research projects in universities, institutions and international laboratories. Sir Sam returns to the academic world in October and although feelers have been put about for several months and several people have been approached through the Department of Education and Science, it seems that no one has yet given a favourable reply.

The job is not without its attractions, because even in these days of cash limits it is still possible to pursue modest initiatives rather than simply shore up the *status quo*; indeed in some ways the power may be greater than before in such matters as postgraduate education, where the universities certainly take more note of SRC's influence and views than in more affluent times. The chairman's job could not really be said to be a post with great appeal to those who have already made their name on the scientific-political scene; strange, then, that one or two of those to whom early feelers went out were people exactly in this category. Maybe commonsense has prevailed—there are several others now being mentioned who have still a name to make; it is surely desirable that with so few scientists privy to the London political scene an imaginative choice should be added to their ranks.

The problem confronting many in this category, however, is what to do after a spell at SRC. If a professorship is resigned at the age of 45, will another professorship or university post conveniently appear at 50? If such prospects look bleak, is it possible to keep in touch with the academic world by spending a day or two every week back at the bench and giving SRC three or four days a week? If the chairman finds public service appealing, will opportunities open up for a now-experienced scientist-politician to be employed in ways that are not trivial? Would a chairman with appropriate leanings be able to find a satisfying post in industry?

That these questions have to be posed seriously and are undoubtedly running through the minds of several people interested in this appointment shows how depressingly rigid career structures can be in Britain for senior people of high competence. The last thing that those of ability should have to do is to cling to tenured posts for fear of loss of income or serious erosion of pension; the country surely needs such people to circulate freely and to be able to put their well-experienced minds whole-heartedly to new tasks, probably administrative more than scientific, right into their sixties. There is a serious danger that by consistently failing to encourage free flow between industry, government and the academic world, Britain is wasting much of its senior intellectual resources; the appointment at SRC is just one instance of the sort of dilemma that faces those who genuinely wish to be mobile.

If a top British post in science policy is discussed discreetly by a charmed circle and names are mentioned only on promises of infinite discretion, to preserve the sensibilities of candidates, no such inhibitions seem to surround the procedure of appointing a Presidential Science Adviser in the United States. For weeks before the appointment is made names are quite openly mentioned as the pros and cons of various candidates are widely discussed. The assumption is, presumably, that aspirants to life in the public eye had better get used early on to being talked about, and that it is better to search out any rational opposition to candidates while there is still time.

Which is the better way of doing things? British concern to protect the individual or American wide exposure? Obviously the question cannot be answered in isolation from recognition of a totally different political and even cultural environment. But even so, there do seem ways in which the appointment of a few of Britain's top science-policymakers could be coupled with much more open consultation, so that the announcement does not come as a complete surprise to virtually all scientists and the name is not greeted with expressions of complete ignorance. This is a matter on which the Department of Education and Science should try to force the pace—they would find mild dissatisfaction with the present system of appointments and a general enthusiasm for more open government amongst scientists. ☐

European energy: where now?

Chris Sherwell looks at the immediate prospects for European energy policy with Britain in the Council chair

ASK almost anyone about energy policy in the European Community and the response, when it is not a blank stare, usually reflects deep-seated disillusion. There is no energy policy, some will imply, and there won't be one that is coherent until someone decides how much of what fuels will be needed for what purposes at which points in the future. Others say that only a policy of fully developing all possible sources of energy will fuel Europe's future growth—which, they may add, is what it is all about.

One thing is certain: the future, like nostalgia, is not what it used to be. Hence the gloom. The OECD's recent *World Energy Outlook*, for example, projecting supply and demand to 1985, pointedly urged industrialised countries to adopt more vigorous energy policies. The EEC experience certainly doesn't augur well. Francois Xavier-Ortoli, the outgoing president of the European Commission, said as much when he spoke disparagingly of the "feeble" European response to calls for a common energy policy. His successor, Roy Jenkins, stressed only last week the need to develop a coordinated policy. And the outgoing EEC Energy Commissioner, Henri Simonet, has said that EEC governments have accomplished "virtually nothing" on energy.

The theme is not always so general. Guido Brunner, the Research Commissioner who has now also taken on the energy portfolio, appealed to Britain a fortnight ago to shift position on the specific issue of the proposed Euratom loans plan; he wants a move on the sensitive issue of a minimum safeguard price for oil (msp) as well. Simonet himself has said that until the Community rids itself of "the ball and chain" of a floor price and gave a go-ahead on Euratom loans it would not be able to develop a policy for boosting alternative energy sources.

Dispelling the log-jam

Now there are hopes that the log-jam may begin to be dispelled. Mr Brunner's appeal followed a series of meetings held in various European centres by Mr Anthony Wedgwood Benn, the UK Secretary of State for Energy, who now holds the chair of the Council of Ministers because it is Britain's turn under the six-month rotation scheme which the Community operates. Mr Benn's purpose was to learn the objectives of EEC members over the period until July (when Belgium takes over), and to discuss the ways the Council worked.

The date of the first Council meeting under Britain's chairmanship is tentatively set for 4 April. The issues it confronts extend beyond the msp and Euratom loans into the broad nuclear field and into the area of fusion and the Community's JET project as well. On all these matters progress will have been hamstrung for a year to eighteen months by the time the Council meets.

Mr Benn freely acknowledges that the Community has been unsuccessful in energy policy thus far. In an exclusive interview with *Nature* last week, he described the approach as "too scholastic", with too many people in offices with computer forecasts operating too much from the centre: officials should be made more responsive to politicians, whose shoulders must bear more responsibility.

In Europe, he says, "everything becomes a package deal" and he doesn't want a "haggling relationship". Rather, he wants a realistic one in which the needs of each country, which aren't identical, are taken seriously. The aim is to "harmonise interests", but there is no point in pillorying a country because it can't move. There should be a search for areas of common interest on which people could build a consensus.

Mr Benn says this view is shared by his European colleagues, being more open, more "discussive" and a move away from judging policy by the number of regulations or directives it produces. In energy matters decisions were not always clear and could not always be taken quickly. No policy sticks, he says recalling the Concorde experience, if it isn't also acceptable to public opinion. An energy policy which makes sense will be put together "bit by bit". And it will fall "far short of any 'federal control' of European energy; I think that is absolutely out".

It is on these lines that Mr Benn wants his Council presidency to be judged. How any success he achieves will manifest itself in the realm of decisions, though, is another matter. He is reluctant to put priorities on the solution of the many long-standing energy questions facing the Council this year: "We'll have to make progress as and when we can", he states, "and I don't want to set myself a target for a particular decision and then have every meeting which doesn't quite realise it written off as a failure".

Keystone

Mr Benn: breaking the log-jam?

Range of problems

A glance at the topics due to come up during Britain's presidency reveals the problems. They range from the barely answerable, like the rational use of energy, to the arguably unresolvable, like the protection and promotion of investment. It is the latter question which focuses upon the highly controversial issue of the msp, the background to which highlights the sort of difficulty the EEC faces.

Irrelevant symbol or careful calculation, the msp's purpose is to make investment both in the North Sea and on alternative energy sources worthwhile. Britain thinks the msp was agreed by the Community at the end of 1975 when Britain dropped her claim, as EEC's only energy exporter, to speak independently of the Community at the upcoming 'north-south' conference. And most members of the International Energy Agency (IEA), the OECD agency based in Paris, have agreed on the idea. But France—not a member of the IEA because, in her view, it encouraged confrontation with the major oil producers—has firmly resisted an msp, insisting, like Mr Simonet recently, that only an investment guaranteeing mechanism was agreed.

No one disputes that the msp issue is politically linked to the issue of the Euratom loans, a scheme under which construction of nuclear power stations might go ahead more fluidly by permitting a Community contribution. Germany and France, with their large nuclear programmes, would both benefit by it. Mr Benn himself says he was mandated twelve months ago to agree to a package in Brussels involving both these items. But there was no progress on the msp. EEC countries agreed in principle to the loans scheme, but Britain refused to sanction its implementation without agreement on the msp, even though she had no objection to it and actually stood to benefit by it. The two matters have remained inextricably linked ever since.

There is a parallel linkage in the

EEC Research Council, now chaired by Mr Gerald Kaufman from the UK Department of Industry. Its inability to agree on a site for JET, the Community's fusion project, has held up implementation of the four-year joint research programme, most recently because of French insistence that the site be at CERN if not in France itself. The two main contenders are Garching in Germany and Culham in Britain.

The possibility that, with the appointment of Mr Brunner as both Energy and Research Commissioner, these two sets of linked issues might themselves become linked is not being talked about openly. But Mr Benn, while pointing out the division of responsibilities between the two Councils, does stress that he is taking a close interest in the subject and affirms that the project is not dead. He could conceivably argue that JET is now to be seen as an energy matter rather than a research project. But for the moment he merely continues to emphasise Britain's view that the Culham site is the most suitable.

JET domino

In a sense JET is a vital domino in the efforts to stimulate some movement on European energy. Britain for one might find it more difficult to posture over certain aspects of policy if she won JET, although she is highly unlikely to relinquish her self-styled role of remaining Europe's energy equivalent of a 'bread basket' by surrendering sovereignty over North Sea oil and gas.

Yet this idea has been publicly canvassed. It was Mr Simonet's view at the end of his tenure that Britain should invite other EEC members to join in North Sea exploitation in return for the guaranteed European market she wants even if prices fall: in other words, access to the goods for access to the market. Mr Benn is fiercely resistant to that suggestion.

He also resists the notion of dealing with one type of energy investment, in nuclear power for example, without dealing with support for other types. To him there is no trade-off to be made: if Europe is to reduce its dependence on imported energy to some 50% by 1985—its intended (if receding) goal—all energy investment must be stimulated.

But even while the haggling goes on over the msp and Euratom loans, the fact remains that Europe will still be the world's largest energy importer in 1985. That, indeed, is why some people say that Europe's nuclear policy, of which the Euratom loans are just one facet, is the most crucial aspect of its energy policy and one which simultaneously offers the best prospect of European cooperation.

The Commission is concerned that the projected nuclear future is now a mere shadow of what it was: the optimistic forecasts of 200 GW of nuclear power by the mid-1980s were reduced to 160 GW some time ago, and according to Simonet the Community would now be lucky to reach 90 GW by then. The problems here, however, stretch beyond mere construction of nuclear power stations.

The EEC countries, all members of Euratom, have so far failed to resolve the now long-standing problem posed by France, which has not signed the Non-Proliferation Treaty, regarding the arrangement for IAEA inspectors to check nuclear installations. France fears IAEA safeguards will be imposed without her consent; her Euratom partners fear she will escape the safeguards they face. One by-product: without agreement on safeguards, a threat to future supplies of enriched uranium from abroad—at a time when at least one of the two European enrichment consortia, Urenco, is facing increasing difficulty.

The issue of reprocessing is still to come on the European front, although reassessment of the matter by the new Carter Administration could have implications for any European intention to reprocess spent fuel on an international basis. Mr Benn hopes to go to the United States to discuss such matters while Council president, although as a UK minister. But the subject of nuclear fuel supply and the nuclear fuel cycle is only slated to come up in the Council in the second half of 1977.

The work programme for Britain's half of the year includes the subjects of oil refining policy, security of oil stocks, the stockpiling of coal and the use of it in power stations. The Council under Mr Benn may not manage to embrace all these matters as well as make progress on the immediate decisions in the two meetings provisionally planned for April and June.

Past experience of the Council certainly does not offer a happy prognosis. But perhaps the man temporarily at the top can remedy that and confound his critics at the same time. It would indeed be ironic if it was Mr Benn who finally did most to promote European cooperation. That is what is now needed. Matters have reached the stage when the degree to which the old Europeans take Britain seriously over energy policy, and *vice versa*, is now to be determined. ☐

USA

An unprecedented debate

Colin Norman reports from Washington on the continuing discussions about genetic manipulation experiments in the United States

AFTER seven months of intense and at times acrimonious debate, the City Council of Cambridge, Massachusetts, last week lifted a moratorium it had placed on some uses of the controversial recombinant DNA genetic engineering technique at Harvard and Massachusetts Institute of Technology. Acting on the advice of a citizens' review board, the council has decided to allow the research to go ahead in Cambridge under strict safety controls and under the watchful eye of a committee of the city's residents.

While the debate in Cambridge has been coming to a resolution, however, it has been picking up momentum in many other parts of the United States. A debate without precedent, it is leading in many cases to an unusual degree of citizens' involvement in the conduct of academic and industrial research, and in the view of many could have a profound influence on future relationships between universities and their surrounding communities.

The following are some of the more important developments which have occurred over the past few weeks.

● **New York.** Legislation to control recombinant DNA research throughout New York state is expected to be introduced into the state legislature this week.* If passed unchanged, it could result in the most stringent controls so far adopted anywhere in the United States.

The legislation is a direct result of a public hearing called last October by the State Attorney General's Office, and the bill was drafted by Deborah Feinberg, an aide in the Attorney General's environmental health bureau. The legislation would essentially require that all academic and industrial research laboratories planning to conduct recombinant DNA experiments must apply for a licence from the state Department of Public Health. The Commissioner of Public Health would be empowered to draft state-wide regulations, based on the NIH guidelines but with additional enforcement provisions. Health monitoring of laboratory personnel would be required, for example, and applicants for licences would have to describe their proposed monitoring procedures. The health department would also be given power to inspect all facilities conducting recombinant DNA experiments, to ensure that the regulations are being followed. Violations could result in a fine and suspension of the licence.

In a 60-page report accompanying the bill, Feinberg recommends that most recombinant DNA experiments should be conducted in moderate containment laboratories (P3 laboratories under the terms of the NIH guidelines), at least until the potential hazards have been better evaluated. "If it then seems safe and appropriate", she suggested last week, "they could be lowered to P2 or P1". The report is advisory, however; the bill requires the health department to set containment levels for classes of experiments.

- **California.** Two committees of the California state assembly, the health committee and the committee on energy, environment and natural resources, have been holding public hearings on recombinant DNA at which a number of prominent opponents and proponents of the research ("the recombinant DNA travelling road show", one wit has described them) testified. A bill, drafted by Marc Lappé, an official in the state health department, is under consideration by the committees, but it has not yet been introduced into the legislature.

Lappé said last week that he expects the legislature to approve legislation later this year which would at least make the NIH guidelines applicable to all facilities conducting recombinant DNA research in California. Like the New York legislation, the California bill is also expected to include provisions for health monitoring of laboratory workers and enforcement by the state government. In addition, Lappé said that the bill now under discussion would require principal investigators to obtain informed consent from other laboratory personnel taking part in recombinant DNA research.

- **Cambridge.** The widely-reported debate over recombinant DNA research in Cambridge is largely responsible for sparking the interest of other city and local governments in the issue. The city council last July imposed a three-month moratorium (later extended to seven months) on P3- and P4-level experiments at Harvard and MIT, and established a committee of Cambridge citizens to recommend the conditions, if any, under which such research should be permitted in the city. Last month, the committee suggested the P3-level work should be allowed to go ahead in accordance with the NIH guidelines, but with some added precautions. All P3 experiments should be conducted on crippled microorganisms, for example, there should be health monitoring of laboratory workers, and a committee of Cambridge residents should be established to oversee the work, to ensure that the regulations are followed.

Last week, the council rejected, by a vote of 6 to 3, a resolution proposed by Mayor Alfred E. Vellucci to prohibit all P3- and P4-level experiments in Cambridge, and it then voted to implement the committee's recommendations. The vote, which essentially incorporated the recommendations into city ordinances, was widely regarded as an acid test of public acceptability of recombinant DNA research.

- **Princeton.** Events at Princeton University provide a unique, and in some respects, exemplary record of cautious debate and discussion before proceeding with recombinant DNA research. Nobody at Princeton was involved in developing recombinant DNA techniques, and thus no experiments were under way there before the national debate began. Last year it was decided no recombinant DNA experiments should be permitted at Princeton until the matter had been fully discussed.

Last September, the university established a special biohazards committee, under the chairmanship of ecologist Robert May, to recommend a policy for the university. The committee, which consisted of three biologists and seven people from other disciplines, unanimously approved a report last December which essentially states that the research should be permitted at Princeton, subject to controls more strict than the NIH guidelines. It suggested, for example, that no P4-level work be permitted, that the NIH guidelines be used as a minimum set of requirements for containing modified microorganisms, and that any P3 laboratories built at Princeton should meet more stringent standards.

The report then went to the University Research Board, which endorsed it unanimously and passed it on to the university's President. He took it to the Council of the Princeton University Community, a group consisting of undergraduates, graduate students, faculty and alumni, which also endorsed it unanimously. The President, William G. Bowen, intended to take the report to the university's trustees, the top governing body, at the end of January, but in the meantime, the local community was brought in. A public meeting organised by officials from the university and from the local township and borough was held to discuss the report, as a result of which a citizens' committee has been established to review the proposals. Bowen is expected to await the findings of that committee before taking the report to the trustees.

- **San Diego.** After the debate erupted in Cambridge, Mayor Pete Wilson of San Diego invited officials of the University of California at San Diego to meet with him to discuss their plans for conducting recombinant DNA experiments. They informed him of their intent to construct a P3-level laboratory in the new medical school, and invited an aide to the Mayor, Mike Madigan, to sit on the university biohazards committee. Subsequently, the city also established a citizens' committee to review the matter. The committee conducted about six months of hearings, taking testimony from a variety of people from around the United States, and it issued a report last month which is expected to be debated by the city council in the next couple of weeks.

The committee intentionally steered clear of recommending whether or not the work should be allowed to proceed at the university, since the city has little formal jurisdiction over the university. It recommended, however, that more citizens be brought on to the biohazards committee, urged the university to disseminate information on its plans for recombinant DNA research, and suggested that health monitoring of laboratory workers be undertaken.

- **Ann Arbor.** An intense internal debate took place at the University of Michigan last year over plans to conduct recombinant DNA research there. The university planned to construct P3-level facilities for recombinant DNA research, and it established a committee to review the biological hazards and a second committee, consisting of non-biologists, to look into the social and philosophical implications.

After an extended debate, the committee of non-biologists recommended, with one dissent, that the university should go ahead with its plans, and the Board of Regents, the university's governing body, subsequently endorsed the recommendation by a vote of 6 to 1.

- **Bloomington.** A public hearing was held last year by an environmental committee appointed by the Mayor of Bloomington, Indiana, to review plans by the University of Indiana to construct a P3 laboratory for recombinant DNA research. The council has so far taken no action, however, and an aide to the Mayor said last week that city officials plan to keep an eye on events, but no city-level regulation is anticipated at present.

- **The federal government**. Much of the concern which has been expressed

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An oversight meant an error in "Carter's energy problem" (3 February, page 398–399): $31,400 million and $42,000 million should have read $314,000 million and £420,000 million, and 100 million and 300 million should have read 1,000 million and 3,000 million. Our apologies.

in state and local debates on recombinant DNA research stems from the fact that the NIH guidelines do not apply to industry, and are not backed by any enforcement mechanism. In that regard, a little-known committee, established last year by President Ford, could prove influential. The committee, which consists of representatives of a variety of federal agencies, is looking into ways to extend the NIH guidelines to cover all recombinant DNA research in the United States. The committee, which meets in closed sessions, is expected to issue recommendations in mid-March.

According to one participant, a broad consensus has been established in the committee that the NIH guidelines should be transformed into enforceable regulations, but it seems that no existing agency has the power to enforce them. For obvious reasons, NIH doesn't want to be placed in the position of both supporting and regulating the research, and the committee is now debating whether to recommend that several regulatory agencies be given the responsibility jointly, or whether to recommend that Congress should pass legislation to give the authority to a single agency. The basis of the regulations, in any case, will be the present NIH guidelines.

In the meantime, a bill was introduced into both the House and the Senate last week to establish a federal licensing scheme for recombinant DNA research, which would apply to both industrial and academic research. Drafted by Senator Dale Bumpers of Arkansas, the legislation is designed to enforce the NIH guidelines as a bare minimum. When he introduced the bill, Bumpers said that if he had his choice, "there would be a moratorium declared right now on all recombinant DNA research". Senator Edward Kennedy's health subcommittee is expected to hold hearings on the bill early in April, after the inter-agency committee has issued its proposals.

Underlying a good deal of the concern is a deep suspicion of what industry is intending to do with recombinant DNA technology, and those suspicions have surfaced dramatically in the past couple of weeks following the publication of a proposal by the Department of Commerce to expedite patent applications involving recombinant DNA techniques.

The proposal, drafted by Assistant Commerce Secretary Betsy Anker-Johnson, would require patent applicants to state that they will abide by the NIH guidelines, though they would be permitted to "include an explanation of any deviations (from the guidelines) considered essential to avoid disclosure of proprietary information". That exemption has attracted criticism because it would allow industrial companies to proceed with their research without notifying anybody.

Such criticism last week prompted Joseph Califano, the new Secretary of Health, Education and Welfare, to ask Secretary of Commerce Juanita Kreps to withdraw the proposal. Dr Anker-Johnson pointed out in a telephone interview last week, however, that the proposal in fact forces industry to disclose more information than it is required to under existing regulations. Further disclosure could result in loss of patent rights in many countries which preclude the award of patents on processes for which there has been any prior disclosure. ☐

CANADA

Guidelines recommended

Canadians are now discussing guidelines for recombinant DNA experiments. David Spurgeon reports

An ad hoc committee of Canada's Medical Research Council has presented its recommendations on guidelines for handling recombinant DNA molecules. The guidelines are similar to those adopted by Britain and the United States, although they are designated differently, but they go further in that they include guidelines also for experiments involving animal viruses and cells.

The committee's report was made public recently in a press conference held by the MRC, which is the federal granting agency for medical research in the universities. If adopted, the guidelines will apply to all MRC grantees, but not to industry. A federal interagency committee has been studying the question as it applies to industry. Dr G. Malcolm Brown, the MRC president, said the ad hoc committee's recommendations would be discussed at a meeting of the council near the end of February, when they could be approved, rejected or modified It is considered almost certain, however, that the council will accept them.

The recommendations were drawn up by a group that included researchers in the appropriate fields and also a member of Queen's University's law faculty. The committee also included a member of the Defence Research Establishment, a member of the University of Alberta's pediatrics department and an observer from the National Research Council.

The Chairman of the committee was Dr Louis Siminovitch, chairman of the department of medical genetics at the University of Toronto and geneticist-in-chief at the Hospital for Sick Children. Dr Siminovitch was influential in the MRC decision to include animal viruses and cells in the committee's terms of reference.

At present, no research involving recombinant DNA is being conducted in Canada, but some experiments have been proposed. A total of one-sixth of MRC's grants could be affected by the proposed guidelines, totalling about $8–10 million a year, because of their effect on animal viruses and cells.

The earliest the recommendations could be put into effect would be April 1, according to Dr Brown, but Dr Siminovitch said many precautions have already been introduced. If the guidelines are approved, the MRC will insist that all recommendations for laboratory safety are carried out before granting funds. The mechanism for enforcing guidelines is thus similar to that adopted in the United States, though different from that in Britain.

The guidelines themselves provide for six levels of containment, ranging from a statement of good microbiological laboratory practice (level A), plus suitable containment measures for agents of no known or potential risk, to requirements for very hazardous materials, such as the virus that causes lassa fever (level F). The technique and equipment described cover all aspects of laboratory research, including the design of laboratories, treatment of effluents, protective clothing and handling of animals.

In the section on recombinant DNA molecules, biological containment methods involving the use of strains of viruses and bacteria that have been altered so that they will not survive outside the laboratory, are included. In the section on cells and viruses, viruses known to cause infectious diseases, and those known or thought to be associated with cancer, are classified according to risk on the basis of their infectivity for man, and also for animals, the severity of the disease they cause, and the range of animals they can infect. Various factors, such as the type of cell, culture conditions, and the manipulations involved, are used to assign the various experiments with animal cells in culture to the containment levels described. Three levels of responsibility are described: the principal researcher, the institution where the work is performed, and the MRC. The chief responsibility lies with the principal investigator.

In its report, the ad hoc committee attempted to relate its proposed standards to those of the US National Institutes of Health and the UK Working Party on the Practice of Genetic Manipulation. "Level A requires no special design features beyond those suitable for a well-designed laboratory," the report said. The level is similar to the NIH's P1, but less stringent than the UK's Category I.

Level B requires a ventilated cabinet and is similar to the NIH P2 and the UK Category I. Level C requires a segregated room or cubicle from which all air is exhausted directly to the outside or is recirculated after passage through a high efficiency particulate air filter. In addition, all air exhausted from protective hoods must be passed through a HEPA filter. This level is more stringent than the NIH P2, less stringent than NIH P3, and similar to UK Category II.

Level D requires a directed airflow through the entire laboratory and thence directly to the outside. All exhaust air must be filtered through a HEPA filter. This level is similar to NIH P3 but less stringent than UK Category III. Level E requires the addition of an air lock, a Type III biological safety cabinet, an emergency plan and alarm system, and a safety cabinet outside the laboratory area. It is more stringent than NIH P3 and similar to UK Category III.

Finally, Level F calls for an isolation unit geographically separate from other areas. This separation may be by means of a separate building or by a separate air system in a multipurpose building. It is the equivalent of NIH P4 and UK Category IV.

A number of experiments with uncharacterised or partially characterised DNA are forbidden at present:

- The deliberate creation of recombinant DNA expected to make harmful products, such as plant pathogens of increased virulence.
- The deliberate release into the national environment of an organism containing recombinant DNA.
- The use of recombinant DNA to transfer drug resistance to microorganisms not known to acquire it naturally, if such transfer could compromise the effective use of the drug in human or veterinary medicine or agriculture.

Other experiments are to be individually assessed by the MRC to determine whether they should be allowed to proceed and, if so, what containments are necessary. These include etiological agents that may pose a special hazard because of particular reasons (that is, that they do not occur in Canada); plants, animals or insects that are either poisonous or whose entry into Canada requires a special permit because of pathogenicity; and all viruses that require containment levels above B.

The report pointed out that guidelines would require periodic review in the light of new information, and that there would still be experiments for which guidelines do not provide explicit containment requirements. For these reasons, the committee proposed that the council set up a standing committee to review the needs and advise the council. □

BRITAIN

Windscale: no escape

No development connected with the Windscale plant operated by British Nuclear Fuels Ltd (BNFL) now escapes national headlines. Chris Sherwell outlines some of the latest problems

WINDSCALE remains in the front line of an increasingly controversial debate over nuclear power in Britain. What encouragement there was last week for BNFL came in the House of Commons, when at the end of a second reading debate on the Nuclear Industry (Finance) Bill a vote was recorded of 196 to 22 in favour. Among other things, the bill provides for an increase from £75 million to £300 million (£500 million by order of parliament) in the limits of the capital which BNFL can raise with government backing

BNFL is reluctant to place any interpretation on the vote for the bill or on its content, but late last year, when expansion at Windscale came under heavy scrutiny, Mr Con Allday, BNFL's managing director, did draw encouragement from the support which he said the existing guarantees represented.

In the House last week, Mr Anthony Wedgwood Benn, the Secretary of State for Energy, implied that the bill was at least a case of prudent preparation. Although he was at pains to stress that the authority provided by the bill would not become operative without planning authority for the expansion, he said it was important that the company should be able to keep open with its customers the option of undertaking reprocessing business should that authority be forthcoming.

On both these aspects there have been some further developments recently. It was just before Christmas that Mr Peter Shore, the Secretary of State for the Environment, announced that BNFL's application to build its proposed oxide fuel reprocessing plant would require the sanction of a public inquiry. Mr Shore also indicated that such a procedure would be unlikely in respect of applications for Magnox fuel facilities and for proposed research projects.

BNFL has since submitted a separate application for the latter expansion to Cumbria County Council, but by last week had yet to announce that it proposed to go ahead with the oxide plant application.

On the related matter of the need for BNFL to keep its options open with its customers, the possibility has now emerged that, when it makes its application, BNFL will split it in two, one asking for permission to build the reprocessing plant itself, and the other seeking permission to construct ponds in which oxide fuel might be stored pending reprocessing. Whether the latter proposal could be sanctioned without an inquiry is not clear, since no one is actually admitting that it is under active consideration. But in the House Mr Benn did manage to give a hint on the matter.

Responding to a question on the building of storage ponds, he said that it was necessary for the company to take discussions to the point of concluding provisional contracts, and that he was having discussions with BNFL "about procedures along these lines". It is thought that this might be a way for BNFL to give additional assurances to the Japanese, at the moment the most important overseas customers. A high level delegation from Japan was in Britain last week for talks with the Departments of Energy and the Environment, with BNFL and with the Cumbria County Council.

Whether the whole issue remains one for the relevant British and Japanese authorities alone, however, is another question. The United States, as supplier of nuclear fuel for stations in Japan and other countries, is in a position to exercise a veto over what happens to spent fuel earmarked for reprocessing.

BNFL are not prepared to comment on this authorisation procedure, which derives from the requirement that any transfer of fissile material of US origin between two countries outside the USA needs the approval of the US Energy Research and Development Administration. US policy thus far has been to authorise the transfer of spent fuel for reprocessing, but the UK Department of Energy says it "may well be" that as part of the US review of non-proliferation policy "further consideration will be given to the conditions under which authorisation is given". According to the *Times*, negotiations between British and the USA on the matter have made little progress so far.

Important as this is, though, the most immediate of Windscale's problems last week appeared to be the continuing stalemate it faced in an industrial dispute which began on 25 January and which coincided awkwardly with the Japanese delegation's visit. Changing room attendants went on strike over a claim for increased allowances for working in an active area. Other workers were laid off as a result, and the strike spread, eventually affecting some 3,000 workers. After 11 days, during which reactors had been shut down and special safety procedures adopted, workers rejected a peace formula; last week no further efforts were being planned to solve the dispute, which was costing BNFL some £14,000 a day in lost revenue.

Meanwhile, worries over the health of workers at Windscale have revived. The deaths of two Windscale workers apparently suffering from cancer-related diseases have been the subject of separate inquests; open verdicts were recorded. Difficulties over secrecy being faced by BNFL lawyers handling two other cases, involving families of Windscale workers who died of cancer-related diseases, are thought to be partly responsible for last week's publication by the National Radiological Protection Board (NRPB) of a paper by Dr G. W. Dolphin, its Assistant Director (Research and Development).

The long-promised paper is substantially the one produced in September 1975 comparing the numbers of observed and expected deaths at Windscale from various cancers. It concluded that the difference was insignificant, but was much criticised, notably by the Flowers Commission on nuclear power and the environment, particularly in being limited only to those actually employed at Windscale.

With the aid of an independent consultant epidemiologist, Dr Peter Smith, BNFL is now to conduct its own two-year study of all 20,000 workers who have been employed at Windscale for any period of time in the past. Its findings will be lodged in a registry of radiation workers which the NRPB has established to produce a long-term study of those exposed to radiation. ☐

USSR

The web of 'secrecy'

Vera Rich reports on the case of Dr Naum Salanskii, now facing problems in seeking to emigrate from the USSR

THE prospect that this year's Diamond Jubilee of the October Revolution might be marked by a spirit of amnesty and a relaxation of pressure on dissidents and minority groups is beginning to look less realistic. A vicious attack on Andrei Sakharov in the embassy 'hand-out' publication *Soviet News*, the arrest of members of the 'Helsinki monitoring group' (including physicist Yurii Orlov and science-fiction writer Mykola Rudenko), and now the peculiar case of Dr Naum Salanskii in Vil'nyus, all indicate an intensification rather than a relaxation of pressure.

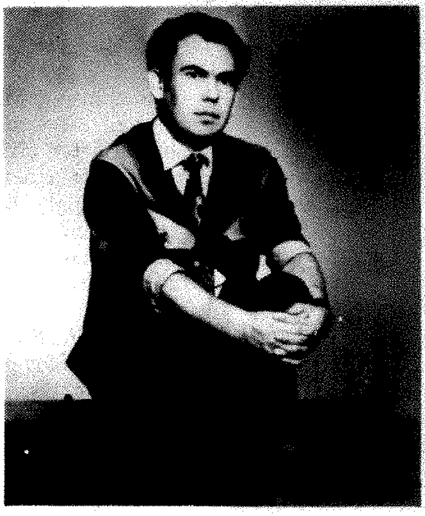
Dr Salanskii, formerly a laboratory head at the Institute of Physics of the Siberian Branch of the Academy of Sciences of the USSR, applied in 1975 to emigrate to Israel and was, according to standard Soviet practice, dismissed from his post. Before leaving the institute, however, he managed to obtain a document signed by the Director of the Institute, Professor I. A. Terskov, which stated unequivocally that "all the works by N. Salanskii were published at home and abroad in journals and other *non-secret* publications".

Dr Salanskii was nevertheless refused permission to emigrate, and on the grounds of his "secret work". With Professor Terskov now retired, Dr Salanskii found it impossible to get the new Director, Professor Pershov, to confirm his predecessor's assessment. While awaiting permission, Salanskii, like so many refusniks, became associated with Mark Azbel's illicit seminars. At the end of December 1976, when the authorities broke up the attempt of the seminar group to hold a three-day symposium on Jewish life in the USSR, several members were held for a time by the police. In Salanskii's case the matter is being taken further.

According to official statements, Salanskii is being investigated on the charge of having "slandered Soviet policy regarding the Jewish minority". The issue of secrecy also occurred several times during interrogations. On one occasion an investigator, Bakuchornis, told him that he had written to Salanskii's old institute enquiring about the alleged secrecy of his work and had already received a reply. On another occasion he was told by an official of the Central Committee of the Lithuanian Communist Party that no such enquiry was ever made nor had a reply ever been received since 'criminal proceedings' had been started. "This seems rather strange", Salanskii commented, in a telephone conversation last week; "first they said that enquiries had been made and a reply received, and suddenly all this has disappeared".

Salanskii is still trying to discover details of the accusation, and has asked the investigator to explain the policy of the USSR towards the Jewish minority which he is alleged to have slandered. According to Salanskii, the investigator said he was not an expert in this field and must look it up in a book. "It seemed very odd to me," says Salanskii, "that he has been working on my case for seven weeks, but still is unable to explain the accusation. As I do not know the essence of the case, I am in the meantime writing the history of my application for emigration [by way of a defence]. If I am taken to court, I will be handed the accusation just before the proceedings begin, so that I cannot prepare a defence."

Salanskii's case is noteworthy for several reasons. Isolated as he is in Vil'nyus, he does not have the moral support which one finds, for example, among the Moscow group of refusniks. And the issue of 'secrecy' has once again raised its bureaucratic head, but in this case it is not only the nature of Dr Salanskii's former work which is considered a secret, but apparently the charges against him too. An early decision has been promised as to whether the charges will be taken to court, dropped, or retained for further investigation—provided, of course, that the outcome of that decision is not itself considered an official secret. ☐



Naum Salanskii

IN BRIEF

Threat to Polish physicist

Miroslaw Chojecki, a Polish physicist and active participant in the unofficial Workers' Defence Committee, has been threatened with arrest. Chojecki, who was dismissed last December from the Nuclear Research Institute at Swierk, has been collecting materials on alleged police brutality and abuses of power in connection with the Radom and Ursus disturbances last June. On 12 February, he sent a memorandum to the Sejm (Parliament), to the Council of State, and to leading journals demanding that the Sejm should appoint a committee to investigate these abuses. Three hours later he was called to the office of the Chief Prosecutor and told that unless he withdrew the memorandum he would be charged and arrested.

The Workers' Defence Committee, set up last September, includes a number of scientists. Among the many recent letters of protest sent to the Sejm, one is from 13 scientists, another by 170 intellectuals, including some 30 scientists, and a third is from 28 professors, 14 of whom are either members of the Polish Academy of Sciences or else working at Institutes of the Academy.

Science adviser tipped

More than three weeks after President Carter's inauguration, vacancies still abound in the federal science agencies, and the most important post, the president's science adviser and head of the Office of Science and Technology Policy, was the only top White House job unfilled by the end of last week. There have, however, been some recent signs of movement. Several candidates were said to be under serious consideration for the science adviser's post last week, and the leading contender was rumoured to be Frank Press, head of the Department of Earth and Planetary Sciences at MIT.

Well regarded in science policy circles, Press was a member of the president's science advisory committee and a delegate to the test ban talks in the early 1960s. He is now a member of the national science board and chairman on the National Academy of Sciences' Committee on Scholarly Communication with the People's Republic of China. Though White House sources cautioned last Friday that Press's appointment was not cut and dried, an indication of high level interest is the fact that Press met with Carter for about half-an-hour last week.

Minister's vaccine statement

Ten days after an announcement that the UK Health Service Commissioner

Hard selling bugs

BACKGROUNDER

"Bugs by the bushel" read the headline to an item in the latest issue of the Pharmaceutical Society's journal, referring to an advertisement that appeared in *Nature* late last year. The advertisement itself, placed by the Ministry of Defence's Microbiological Research Establishment at Porton Down, was headlined "Bacteria by the Kilogram" (November 11, 1976—Cover 3). The journal had expressed concern that terrorists might acquire dangerous bugs.

A London evening newspaper picked the story up and ran it on the front page. The national dailies, ranging from the *Times* to the *Daily Mirror*, gave it prominence the following day, the *Times* on its front page. A spokesman from the Pharmaceutical Society was quoted as saying that all the bacteria named in the advertisement were very dangerous toxins, no less.

An MP who found the matter "outrageous" arranged to ask a question in the House of Commons about the offer. A junior minister, Mr John Gilbert, duly reported the same day that the sale of bacteria for civil research requirements was not a new departure for the MRE, though the offer by means of advertising was. And the storm seemed to pass.

What was the fuss about? The Pharmaceutical Society journal article focuses strongly on the implications of a particular section of the advertisement. Three words, in fact, saying that the MRE can supply the products advertised "to any destination". This does look worrying when the products include "toxic proteins" and "animal viruses", and when the MRE says, "We also grow new strains for research workers—if you have a requirement please ask if we can help". Small wonder, it seems, that others should latch on to the journal's worry about terrorists.

Mr Gilbert did have some comforting words in the Commons, however. The bacteria weren't dangerous pathogens. They were sold solely for research. And the phrase "to any destination" was there, he said—this, the catch-all British legitimation — to make it clear that export inquiries were welcomed.

Mr Gilbert also assured the Commons that the only bacterial species mentioned which could be conceivably harmful to humans were supplied only to bona fide customers at recognised laboratories or research institutes. They were dispatched in full conformity with international regulations.

The harmless species advertised included *E. coli*, found in the human gut, and *B. stearothermophilus* and *T. equaticus*, which are soil and water bacteria that do not infect man or animals. Researchers need bacteria in large quantities for the extraction of even small amounts of various microbial proteins. The MRE has the time, expertise and facilities to grow them.

Indeed, the MRE has recently expanded its Microbial Research Products Division to meet the growing demand for a wide variety of microbial products, in particular the restriction endonucleases — enzymes which snip DNA at precisely specified sites and which are now essential tools for recombinant DNA work and to generate DNA fragments for cloning. The MRE also has a contract service for researchers needing large quantities of particular bacteria.

What makes the latest development newsworthy is, as Mr Gilbert implied, the *Nature* advertisement. The Ministry of Defence decided last year that it will no longer meet the cost of providing facilities and staff at the MRE for the non-military work it does (military-related work is being transferred to the nearby Chemical Defence Establishment). That means researchers in microbiology in Britain stand to see the only facility of its type go to seed unless it can stand on its feet outside the Ministry of Defence. So to drum up business, the MRE has started advertising.

is to take up the cases of four children said to have suffered brain damage after whooping cough vaccinations, the UK Social Services Secretary, Mr David Ennals, last week told the House of Commons that despite their case he could not consider claims for vaccine-damaged children in isolation ahead of a Royal Commission report on liability and compensation for personal injury.

He expressed concern at the 60% fall in whooping cough vaccinations over the past three years, and added that the government's adviser on immunisation thought the policy of offering whooping cough vaccine should not be changed as the benefits far outweighed the risks.

UK coal report

With the help of continuing high inflation and a disappointing productivity performance the costs associated with expanding coal production in Britain to 135 million tons a year by 1985 have more than doubled from £1,400 million three years ago to £3,150 million. However, the British coal industry's tripartite group, embracing the government, the National Coal Board and the unions, shows no signs of changing the emphasis accorded to coal in the country's energy strategy. In its third report (*Coal for the Future*, free from the Department of Energy) updating progress on the ambitious 1974 "Plan for Coal", the group stresses the importance of research and emphasises the role for UK coal in EEC energy policy.

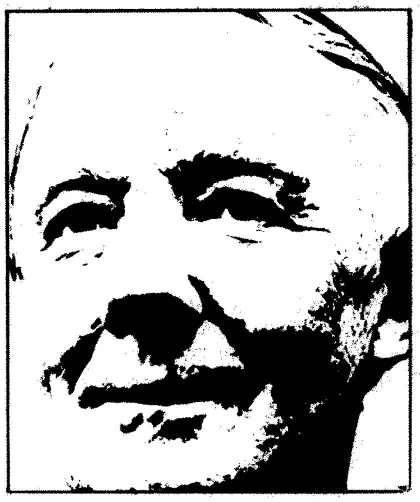
Energy unit set up

The UK Science Research Council has set up a support unit at its Rutherford laboratory to assist the energy research it funds at universities and polytechnics. Known as the Energy Research Support Unit (ERSU), it will also conduct feasibility studies for proposed projects and undertake necessary development work for industry to adopt research programmes.

Orkney ore demo

On the day that Orkney Island council's planning and development committee agreed to recommend the rejection of an application from the South of Scotland Electricity Board to survey for uranium, the island experienced what is believed to be its first-ever demonstration when some 400 people marched in opposition to the application. The SSEB has an agreement with some land owners and may appeal to the Secretary of State for Scotland to overrule the objections. The survey is partly financed by the EEC.

Close to the hedge



KENNETH MELLANBY

THE British landscape of small fields and hedgerows was the product of nineteenth century agriculture. Until recently, our most efficient farms were the most beautiful and provided plenty of cover for a rich selection of our native fauna and flora. British farmers consider that they are the real conservers of the countryside. This view has had official support, for most of the activities of farmers—rooting out hedges, ploughing ancient grass land, the erection or removal of all but the largest buildings—are free from planning controls. Today the whole question of rural land management and conservation needs to be re-examined.

The word 'conservation' has, unfortunately, many meanings. It can refer to the maintenance of the fertility of the soil, to preserving grass or forage as hay or silage, as well as to preserving wild animals and plants. Farmers in Britain are indeed conserving their soil and are not, as some critics suggest, destroying its structure by methods aimed at obtaining high yields today. In fact it is our most heavily cropped areas which are the most fertile; the field which gives the highest yield this year will probably give the highest next year also. Good farming is the best method of building up and maintaining the fertility of the soil.

Unfortunately, however, some recent agricultural developments, though doing wonders for food production, no longer ensure that rural Britain retains much of its beauty and diversity. In the second annual report of the Nature Conservancy Council the dangers of modern agriculture to wildlife are clearly described without any of the exaggerated overstatements which so often detract from the jeremiads of some conservationists. Large fields of clean, weed-free cereals and productive grass leys containing only one or two cultivars are of little value to wildlife. Though the willingness of many farmers to make some compromise by retaining cover on unproductive corners of their land is welcomed, it is clear that positive action by conservationists of all kinds is needed, and that much more money will have to be spent saving marshes and bogs, old grass and other habitats which have no useful place on the modern farm.

The situation is more serious than some of us, including scientists involved in these problems, may realise. Perfectly sound ecological research has suggested that some changes, particularly the removal of hedges, have done less damage to wildlife than is generally imagined. Many birds, for instance, have proved to be remarkably adaptable, and can still exist in substantial numbers when the landscape has been greatly impoverished. The somewhat theoretical 'index of diversity' may not be substantially reduced when many hedges and hedgerow trees are removed. These findings must not be allowed to make people over-optimistic. The fact is that many of the most influential conservationists are ornithologists and as long as birds are not too greatly affected, they have been apt to accept changes affecting plants and other animals. Now birds are useful as delicate indicators of pollution, but the purpose of the rest of the countryside is not only to provide them with living quarters.

Birds may tolerate the removal of many hedges and trees, but the shrubs and trees lost are themselves an important part of the native flora we wish to conserve. An oak tree is not only a roosting place for pigeons and kestrels, and the source of food for some 300 different species of insect; it is in its own right the largest chunk of wildlife to be seen on many acres of farmland. Conservation must devote more attention to preserving the common and everyday features of the countryside and must make the enjoyment of these easily available to our people in general if more public support and funds are to be used to protect the countryside. The conservation of rarities and the support for esoteric ecological studies is not enough.

news and views

Ferromanganese deposits: fast, fast, slow

from Peter J. Smith

FERROMANGANESE deposits on the ocean floor were discovered by the *Challenger* expedition more than 100 years ago and have been objects of scientific study and speculation ever since. In recent years, however, their existence (especially in the form of nodules) has become well known far beyond the scientific arena, largely because of their potential economic importance and the problems this has produced in devising a fair and workable international law governing the exploitation of the seabed. Some oceanographers have expressed resentment that this familiarity has given ferromanganese deposits a popular prestige totally unjustified by their volume in comparison with the volume of all oceanic sediments—a view that would perhaps carry more weight if the renewed interest in oceanic ferromanganese and associated metals had not drawn attention to the importance of hydrothermal processes at oceanic ridges. But nodules, slabs, grains, coatings and intrusions of oceanic ferromanganese remain a fascinating scientific issue quite independent of their economic implications.

Part of their scientific attraction undoubtedly lies in their ability to grow remarkably rapidly under suitable circumstances. On page 596 of this issue of *Nature*, for example, Burnett and Piper report the discovery of a ferromanganese crust which apparently grew at a rate in excess of 800 mm per million years. This figure may be put in perspective by the claim by Scott *et al.* (*Geophys. Res. Lett.* **1**, 355; 1974) that deep sea ferromanganese crusts and nodules generally grow at rates of 1–10 mm per million years. In addition, Burnett and Morgenstein (*Earth planet. Sci. Lett.* **33**, 208; 1976) report that in a forthcoming book Ku will conclude that "both the radiometric and nonradiometric techniques employed to assess . . . rates demonstrate conclusively that ferromanganese nodules of the deep sea grew at extremely slow rates . . . on the order of millimeters per million years."

It is not clear from this reference whether Ku's conclusion will be held to apply to all ferromanganese deposits, in which case the validity of claims for higher rates must presumably be denied, or whether it will be a more general statement applicable only to the majority of deposits. Either way, many claims for higher rates have certainly been made. One of the earliest was by Pettersson (*Medd. Oceanog. Inst. Goteborg, Ser. B* **2**, 1; 1943) who determined a growth rate of 1,000 mm per million years for a manganese nodule by measurement of its radium content. Using the same method, Von Buttlar and Houtermans (*Naturwissenchaften* **37**, 400; 1950) later found rates of 600–1,300 mm per million years for several other nodules. And more recently Scott *et al.* have reported growth rates of 130–250 mm per million years for a manganese oxide crust, and Moore and Vogt (*Earth planet. Sci. Lett.* **29**, 349; 1976) have obtained rates of 1,000–2,000 mm per million years for similar crusts.

Some of the most astonishing rates of growth, however, were discovered by Goldberg and Arrhenius (*Geochim. cosmochim. Acta* **13**, 153; 1958) who reported finds on the sea floor of an approximately 50-yr old naval shell with a Mn–Fe oxide coating almost 30 mm thick and a World War II shell with a coating about 15 mm thick, indicating growth rates of about 60,000 and 100,000 mm per million years respectively. Admittedly these deposits formed in shallow near-continental waters and may thus not be comparable to deep sea deposits. On the other hand, their ages could be inferred directly without recourse to radiometric and other physicochemical methods of dating, and so the determined growth rates are fairly certain, at least to the correct order of magnitude. Unfortunately, this is not so for older ocean floor deposits—a situation which leaves room for doubt about the validity of measured growth rates in general.

Indeed, Burnett and Piper acknowledge the possibility of doubt in showing that different methods of dating lead to quite different estimated growth rates. Specifically, the rate of 800 mm per million years was obtained by hydration-rind dating and by measuring an excess ^{230}Th exposure age; uranium-series dating gave a much lower rate of about 48 mm per million years. Burnett and Piper give apparently valid reasons for supposing the latter rate to be incorrect, thus making a case which Burnett and Morgenstein recently developed at greater length. In the previous study, of four Pacific ferromanganese deposits, two had uranium-series and hydration-rind dates in agreement whilst two produced significant discrepancy. In each of the two disagreeing cases the uranium-series date was the lower, further study suggesting that it was incorrect because of a thorium distribution controlled by processes in addition to radioactive decay.

In spite of such problems, however, it seems likely that when dating methods have been fully assessed and possible sources of error identified, many of the higher (as well as the lower) growth rates claimed for ferromanganese deposition will be substantiated In other words, there is apparently a wide variation in growth rates which must be taken into account in deciding how deposits form. Over the past 100 years many models have been proposed to explain the origin of ferromanganese deposits, including the interaction of sea water with submarine volcanic products, direct precipitation from sea water of land-derived ferromanganese, and the remobilisation of ferromanganese from oceanic sediments or underwater igneous outcrops. But although these processes may play some part in some or all circumstances (for example, direct precipitation may largely account for the high deposition rates often observed in near-continental shallow water), high rates of growth observed in samples from oceanic ridges suggest the existence of at least one other major source.

In recent years this has been identified as hydrothermal activity, which in this context means the circulation of seawater within cracks and pores of hot, newly-formed basaltic crust, the leaching of metals from that crust and the consequent metal enrichment of the resulting hydrothermal fluids. Hydrothermal activity is the most likely candidate for several reasons, not the least of which is the need to invoke it for other purposes such as to explain the distribution of heat flow in the vicinity of oceanic ridges. But more directly, by extrapolating from the East Pacific Rise, Lyle (*Geology* **4**, 733; 1976) has recently estimated the total input of hydrothermal manganese to the world's oceans to be about 9×10^8 kg yr^{-1}, or nearly four times the dissolved load of manganese carried by rivers. Unfortunately, there are no reliable figures available for the detrital input of manganese, and so the total contribution from the continents remains unknown. Nevertheless, by any standards hydrothermal activity is evidently an important source of oceanic ferromanganese. □

How does T4 turn off *E. coli*?

from Christopher G. Goff

IT has been clear for a long time (on the time scale of molecular biology) that bacteriophage T4 can turn off the expression of *E. coli* genes in its host cell, while continuing to direct synthesis of its own gene products (Benzer *Biochem. biophys. Acta* **11**, 383; 1953; Hayward and Green *Proc. natn. Acad. Sci. U.S.A.* **54**, 1675; 1965). This is true despite the fact that expression of T4 genes depends throughout infection on the *E. coli* transcription enzyme and *E. coli* protein synthesis machinery. A satisfactory explanation of this "host shutoff" has been hard to come by. We know that T4 degrades *E. coli* DNA, while protecting its own genome from self destruction by replacing its cytosine with hydroxymethylcytosine, not recognised by T4 nucleases. But host gene products stop appearing well before *E. coli* DNA is broken down. The shutoff mechanism is obviously complex; it may involve more than one of the numerous T4-specified changes which occur in both the transcription and translation apparatus of *E. coli* after infection. Nevertheless two recent papers suggest how one specific T4 protein bound to *E. coli* RNA polymerase may be a major factor in the shutdown of *E. coli* RNA synthesis after infection.

There are four T4 proteins non-covalently attached to *E. coli* RNA polymerase after T4 infection (Stevens *Proc. natn. Acad. Sci. U.S.A.* **69**, 603; 1972). Two of these, the products of T4 genes *33* and *55*, are positive control elements needed for synthesis of the major class of T4 RNA appearing late after infection (Horvitz *Nature new Biol.* **244**, 137; 1973; Ratner *J. molec. Biol.* **89**, 803; 1974). Another of these four proteins, 10,000 daltons in size but genetically undefined, apparently alters or inactivates the sigma subunit of RNA polymerase (Stevens & Rhoton *Biochemistry* **14**, 5074; 1975), at least *in vitro*. The fourth, 15,000 daltons in size, has until now been mysterious; no lethal mutations have defined its gene or its function. But Snyder's lab at Michigan State has recently reported (Sirotkin, Wei & Snyder *Nature* **265**, 28; 1977) that the *alc* mutants isolated last year by Snyder, Gold, and Kutter (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3098; 1976) affect this T4 protein. These *alc* mutants were isolated in a multiply-mutant strain of T4 unable to synthesise hydroxymethylcytosine and unable to degrade normal cytosine-containing DNA. One might expect these phage, whose DNA contains cytosine, to be viable; but they are not, because (it turns out) they cannot make the 'late' class of T4 mRNA. A single additional mutation—in the *alc* gene—permits these cytosine-containing T4 to transcribe late RNA and grow. Snyder's lab now finds that many *alc*$^-$ phage fail to attach normal amounts of the 15,000 dalton protein to *E. coli* RNA polymerase. This suggests (but doesn't yet prove) that *alc* codes for the 15,000 dalton protein. Moreover, they find that several times more *E. coli*-specific RNA continues to be made after infection with *alc*$^-$ T4 than with *alc*$^+$ T4. Here again, more data are needed, but it is possible that the presence of functional *alc* protein prevents RNA polymerase from transcribing cytosine-containing DNA, whether that DNA encodes T4 genes or *E. coli* genes. This hypothesis could explain why no conditional lethal 15,000 dalton protein mutants have been found in normal T4: the protein would not be necessary for transcription of the usual hydroxymethylcytosine-containing phage DNA, and failure to shut off host RNA synthesis would not be lethal either.

How might the *alc* protein block transcription of cytosine-containing DNA? Baralle and Travers (*Molec. gen. Genet.* **147**, 291; 1976) have recently found that *E. coli* RNA polymerase purified from T4-infected cells (and containing the 15,000 dalton T4 protein in question) has a lower affinity for *E. coli* rRNA promoters than does *E. coli* RNA polymerase from uninfected cells. This is true even when both forms of the enzyme are given excess initiation-specificity subunit sigma. The difference is very likely due to the 15,000 dalton protein; the authors have evidence that Stevens' 10,000 dalton T4 protein and an ADP-ribose residue added to the α subunit of RNA polymerase (Goff *J. biol. Chem.* **249**, 6181; 1974) are not responsible. In some *in vitro* conditions which permit normal *E. coli* RNA polymerase to initiate synthesis rapidly at rRNA promoters, the enzyme from T4-infected cells is unable to use those promoters at all. In these conditions, then, the 15,000 dalton protein seems to cause essentially complete shutoff of rRNA synthesis. The big question in any such *in vitro* experiment is, of course, "What are the conditions *in vivo*?"; but certainly the 15,000 dalton (putative *alc*) protein could have a role in host shutoff. Travers has suggested before that RNA polymerase can "flip-flop" between a form active on ribosomal RNA promoters and one or more forms active on mRNA promoters, and that guanosine tetraphosphate (ppGpp), associated *in vivo* with the stringent response, forces RNA polymerase out of its rRNA-specific conformation (*Molec. gen. Genet.* **147**, 225; 1976). In their *Molecular and General Genetics* paper, Baralle and Travers show that the T4 infection has the same effect on RNA polymerase as ppGpp; the nucleotide cannot further reduce the fraction of rRNA made by the enzyme from T4-infected cells, in any *in vitro* conditions. So the *alc* protein may be an allosteric effector locking RNA polymerase in a form inactive on at least some *E. coli* promoters—and conceivably inactive on T4 promoters as well, if they contain cytosine rather than the usual hydroxymethylcytosine.

There are still a lot of questions here, even if Snyder and Travers are completely right. Baralle and Travers' *in vitro* experiments reveal no reduction in transcription of φ80 mRNA from cytosine-containing DNA when the 15,000 dalton protein is present. Would the *alc* protein alone prevent use of cytosine-containing T4 promoters *in vivo*? Mailhammer *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **72**, 4928; 1975) saw a significant reduction of *E. coli* β-galactosidase synthesis in a coupled *in vitro* protein synthesising system when they used RNA polymerase from T4-infected cells; they attributed the apparent shutoff of *E. coli* mRNA synthesis to the ADP–ribosylation of the α subunit. *In vitro*, and presumably *in vivo*, active sigma protein is required for transcription of both *E. coli* and T4 genes. Yet Stevens' 10,000 dalton T4 protein inhibits sigma function *in vitro*; this T4 protein has been largely ignored in the studies mentioned here. Possibly the *alc* protein, ADP–ribosylation of α, and the 10,000 dalton protein all play a part in shutoff of *E. coli* transcription when T4 infects the cell. □

Bacterial adherence

from a Correspondent

THE ability of many pathogenic as well as beneficial microorganisms to attach to a particular surface of their host is one obvious requirement for colonisation and in some cases invasion of that host. The initial attachment of bacteria to the nasal or intestinal mucosa, for example prevent them from being washed away or dislodged by a cough or a sneeze. But surprisingly, little is known about the initial interactions between colonising bacteria and the host tissues. Some recent work suggests, however, that the interactions may be considerably more complex and specific than previously realised and some of the surface molecules involved have been identified.

Since carbohydrate, in the form of glycoproteins or glycolipids, is an important constituent of most cell surfaces, including bacterial and mucosal surfaces, a role for these substances in bacterial adherence has been suspected for more than 20 years. Duguid and colleagues, following an earlier observation of Collier and de Miranda, were the first to show this directly by using mannose to inhibit binding of *Escherichia coli* to intestinal epithelial cells (*J. Path. Bact.* **74**, 397; 1957). A large number of other simple sugars were tested and found to be inactive. The bacterial surface component responsible for adherence appeared only in old non-growing cultures about the same time as filaments appeared. These original observations have now been taken a step further by Ofek, Mirelman and Sharon (see this issue of *Nature*, page 623). They show that in agreement with the earlier results binding of non-pathogenic K12 or B strains of *E. coli* to human cells is specifically inhibited by mannose or mannosides. Binding is also shown to be inhibited by a mannose-seeking plant lectin (concanavalin A) which presumably competes with the bacterial surface component for epithelial cell-surface mannose receptors. These receptors are destroyed by mild periodate treatment of the epithelial cells, which is consistent with the presence of mannose-like receptors, since these groups are particularly susceptible to periodate oxidation. The bacterial component (a lectin) is extracted into simple buffers without detergents and is presumably part of the filaments seen in adhesion-competent organisms, rather than being an integral part of the bacterial envelope. The soluble component, as yet unpurified, agglutinates cells at high dilution and inhibits attachment of *E. coli* to cells. It is a protein or perhaps glycoprotein which is destroyed by trypsin or heat.

The nature of the periodate-sensitive epithelial cell receptors carrying mannose and recognised by the bacterial lectin is unknown. Not surprisingly, mucins present in intestinal tract secretions, which contain little mannose, were found by Duguid and Gilles to be poor inhibitors of bacterial adherence. Other glycoproteins containing more mannose and binding concanavalin A are presumably the functional receptors. Perhaps these glycoproteins are more intimately associated with the mucosal cell surface membrane since ultimately invasion of the mucosal lining (perhaps by phagocytosis) takes place. Alternatively, these later membrane-mediated events in bacterial invasion may involve additional interactions between the epithelial cell surface and other bacterial surface components.

A wide range of *E. coli* strains show mannose-sensitive binding to host cells and to erythrocytes in haemagglutination (Jones & Rutter *J. gen. Microbiol.* **84**, 135; 1974), although not all, even some which produce enterotoxin, are pathogenic. Two *E. coli* surface components appear, however, to correlate exactly with pathogenicity in pigs and calves. These are the K88 and K99 antigens respectively (Jones & Rutter *op. cit.*; Burrows, Sellwood & Gibbons *J. gen. Microbiol.* **96**, 269; 1976). The K antigens are proteins, most probably glycoproteins, since a small amount of carbohydrate is present even in the purest preparations, and can be distinguished from the lectin-like substance by their binding to mucosal epithelial cells in the presence of mannose. Like the lectin, however, their production by bacterial strains always correlates with the appearance of fine branched filamentous projections covering the surface of the cell. Together with the mannose-binding lectin, the K antigens may form a part of the projections and if so are then favourably placed to interact with the surface of the preferred host cell.

So far analogous surface antigens on human enteropathogenic strains have not been identified unequivocally but it is reasonable to assume that such components, in addition to the lectin identified by Duguid and Ofek *et al.*, are necessary for the successful colonisation and invasion of the human intestinal tract as opposed to the normal colonisation of the colon.

Clearly, the relation of mannose-binding lectin and the K antigens in bacterially-induced neonatal diarrhoea is of great interest, since this disease is now one of the major causes of death in small children in developing countries (Sack *A. Rev. Microbiol.* **29**, 333; 1975) as well as being a severe economic burden in livestock production. In the future, more detailed identification of those surface components involved in bacterial adherence could lead to rational schemes for the preparation of broad range vaccines effective in blocking or reversing bacterial attachment. Such vaccines may have advantages over more conventional vaccines which aim to neutralise toxins which are active at a later stage in infection. □

Optical fibres in communications

from a Correspondent

ONE of the consequences of rapid growth in demand for telegraphic communications is a continuing search for more efficient ways of transmitting information over large distances (see *Nature* **261**, 371; 1976). High rates of signalling call for transmission lines with high frequency response; hence our telephone services now make use of microwave devices at very high frequencies of the order of 10^9 Hz. It was inevitable that in the search for ever higher transmission rates electromagnetic waves in the visible range would be considered. With frequencies of the order of 10^{15} Hz, light waves are potentially capable of solving line-capacity problems for the foreseeable future.

One difficulty is that light is so easily absorbed. Line-of-sight transmission through the atmosphere is too unreliable to be of use, and research has concentrated on channelling the light inside fine glass fibres which can be made at relatively low cost and have the flexibility associated with ordinary wires (see for example, Chynoweth *Phys Today* **29**(5), 28; 1976). If sufficiently transparent glasses can be found, these fibres will be the telephone wires of the future. At the recent Solid State Physics Conference in Manchester, UK, glasses having losses as low as 0.5 dB km^{-1} were reported in a review lecture given by W. A. Gambling.

Disordered materials in general and glasses in particular present some of the most difficult problems in solid state physics. Absorption of photons takes place by electrons which are thrust into unoccupied states of higher energy. In crystalline materials transparency results when there is a gap in energy between occupied and unoccupied states which is too wide for the photon energy to excite electrons across, any residual absorption is due to impurities or imperfections in the crystal. In a glass there is no well-defined energy gap and its transparency must be explained in another way; despite the absence of an energy gap there exists a range of energies in which the electronic levels are localised rather than extended over the whole of the material. Transparency in glass comes about because, although there may be empty states of the correct energy to receive an electron which has just been excited from a localised state by a photon, these empty states are almost certainly localised in a different part of the glass and so are not available to receive the electron. Thus a glass may have excellent transparency limited only by scattering from statistical irregularities in density frozen in at the solidification temperature, provided it contains no impurities which themselves can absorb light. Iron and copper are particularly troublesome contaminants and must be reduced to the level of 1 part in 10^9 for really low losses.

To achieve such purity novel methods of glass manufacture are used. In one method silicon tetrachloride gas is reacted with oxygen to deposit silica on the inside of a quartz tube. Dopants can be introduced into the gas to vary the refractive index of the deposit, which is important for controlling propagation of light in the fibre. After sufficient silica has been deposited, the tube is collapsed and the fibre-drawing process begun. Several kilometres can be pulled from a single tube.

Currently it is possible to produce optical cables with low enough losses ($\sim$4 dB km^{-1}) to allow distances of several kilometres between repeater stations, and test lines have already been installed near Ipswich in the UK by the Post Office. Developments in glass technology already in hand should assure the economic advantage of the new method of transmission in the near future. ☐

HPRT mutants

from Dallas Swallow and Sue Povey

MUTANT cells deficient in the enzyme hypoxanthine phosphoribosyl transferase (HPRT) have been isolated from cell cultures of a variety of mammalian species. These deficient cells have been selected, in many cases after mutagen treatment, in medium containing purine analogues such as 6-thioguanine which are toxic to cells containing HPRT. However, the nature of the mutations involved has been the subject of much debate. Whereas some of the mutants can most readily be explained by mutations in the X-linked structural HPRT locus, as an altered HPRT protein has been identified, many are probably regulatory. This was originally suggested by the lack of the expected relationship between cell ploidy and mutation rate (Harris *J. Cell Physiol.* **78**, 177; 1971; Chasin *J. Cell Physiol.* **82**, 299; 1973) and further, by the occasional reappearance of apparently normal rodent HPRT following somatic cell hybridisation between a rodent cell line deficient in HPRT and a normal human cell (Watson *et al. Expl Cell Res.* **75**, 401; 1972; Shin *et al., Nature new Biol.* **241**, 194; 1973; Croce *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 2590, 1973).

In a recent paper Milman and coworkers have examined HPRT in the aneuploid human cell line HeLa, using an elegant technique characterising the enzyme in terms of isoelectric point and subunit molecular size (*Proc. natn. Acad Sci. U.S.A.* **73**, 4589; 1976). The protein synthesised by these cells was labelled with ^{35}S-methionine and cell extracts subjected to isoelectric focusing followed by electrophoresis in sodium dodecyl sulphate. In the two-dimensional autoradiograph obtained, the spot corresponding to the usual HPRT was identified by several criteria including its removal by immunoadsorption using specific anti-HPRT antiserum. This spot was absent in each of 24 6-thioguanine resistant colonies isolated from HeLa after mutagen treatment. One of these $HPRT^-$ mutants, however, produced a new spot, different in isoelectric point but of the same subunit molecular size, and also cross reacting with the anti-HPRT serum. Five independent revertants of this 'missense' mutant, able to grow in MTH medium (containing methotrexate, thymidine and hypoxanthine) which selects for the presence of HPRT, were isolated. All of these, even after repeated cloning, contained spots corresponding to both the usual and the abnormal HPRT proteins. From these revertants further 6-thioguanine resistant colonies could be readily obtained and these had again lost the 'usual' HPRT spot.

If one accepts that the revertants were indeed true clones the simplest explanation of this intriguing experiment is that the loss of HPRT in the 'missense' mutant was due to a mutation at the structural locus, and that the revertants represent activation of some HPRT locus specifying an enzyme indistinguishable from the usual one, but not normally expressed. As HPRT is known to be multimeric (see, for example, Olsen & Milman *J. biol. Chem.* **249**, 4030, 4038; 1974) it would be of interest to examine these revertants using a technique which might demonstrate heteromeric isozymes. The existence of such hybrid forms would confirm that both genes are active in the same cell at the same time.

It is not entirely clear whether HeLa resembles other clonal cultures derived from female somatic cells in having only one active X chromosome. However, if this is the case, then an attractive hypothesis to explain the findings is the reactivation of the HPRT gene on the normally inactive X chromosome. Although many attempts to reactivate the inactive X chromosome in cultured somatic cells have not been successful (see for example Comings *Lancet* **ii**, 1137; 1966; Migeon *Nature* **239**, 87; 1972), good evidence for reactivation of the HPRT locus was obtained in one series of human–mouse hybrids (Kahan & DeMars, *Proc. natn. Acad. Sci U S A.* **72**, 1510, 1975). In this experiment the authors were able to identify both X chromosomes by virtue of one's being present as an X-autosome translocation and also by heterozygosity for the X-linked enzyme, glucose-6-phosphate dehydrogenase (G6PD) as well as HPRT deficiency. HPRT$^+$ clones were selected using HAT medium (hypoxanthine, aminopterin and thymidine). All the clones contained human HPRT, as identified immunologically, and the inactive X chromosome which carried the normal HPRT gene, but not the active X chromosome. The other X-linked enzymes, G6PD and phosphoglycerate kinase, were not detectable suggesting that the derepression was very localised. If reactivation of the HPRT locus on the inactive X chromosome does account for the reappearance of HPRT in the HeLa clones of Milman *et al.*, it would be interesting to test other X-linked loci in these cells.

It has been deduced from family studies that Henrietta Lacks from whom the HeLa line was derived was a heterozygote for G6PD (type BA), although the cell line HeLa is G6PD A (Hsu *et al. Science* **191**, 392; 1976). Thus it would be interesting to test G6PD in these clones to look for the derepression of the G6PD B allele on the inactive X chromosome.

Future experiments may throw some light on the mechanism of the maintenance and possible reversal of X-inactivation. It is unclear whether a similar explanation could account for some of the rodent revertants, since the X chromosome constitution and indeed the sex of origin of the various long-term rodent lines are in many cases unknown. However, it is possible that some completely different phenomenon which might have more general application to autosomal loci, will be found to explain the reappearance of HPRT in the experiments of Milman and others. □

More Luna results

from J. A. Edgington

LAST year's successful flight of Luna 24, the third Soviet spacecraft to return lunar samples to Earth, coincided with the Viking landings on Mars and attracted less attention than it deserved. Its core sample from Mare Crisium is now being examined in Moscow and Western scientists hope to receive some of this material soon. Under a US–Soviet agreement the US has provided 3 g from each of the six Apollo flights, and the Russians have reciprocated with 3 g of Luna 16 and 2 g of Luna 20 sample, out of total sample returns of 100 g and 50 g respectively. In addition, 1 g from each Luna mission was donated to French investigators, and 500 mg to British scientists.

All but about 100 mg of each UK sample was divided between eight investigating teams, and accounts of the separation procedures, together with experimental results from some of the investigators, have recently appeared in *Phil. Trans. R. Soc.* (**A284**, 131; 1977). Luna 16 landed in Mare Fecunditatis in 1970, and Luna 20 in a nearby highland area in 1972; papers by the American and French investigating consortia appeared some time ago (see *Geochim. cosmochim. Acta* and *Earth planet. Sci. Lett.* for April and September, 1973). The present articles, perhaps because of their relatively leisurely publication, provide good examples of small sample techniques and of careful data interpretation.

For example, N. N. Greenwood *et al.* (University of Leeds) have used Mossbauer spectroscopy to estimate the relative abundances of iron-bearing minerals, and their interpretation of the spectra, based on experience with Apollo samples, is somewhat different from that of the Russian workers (for example, T. V. Malysheva, *Proc. 3rd. Lunar Sci. Conf.* 105; 1972) and generally more cautious. However, one feature consistently observed in lunar fines material, a pronounced asymmetry in the pryroxene absorption spectrum, is definitely ascribed by the Leeds group to the presence of extremely small particles of metallic iron, which moreover they have shown to be concentrated in the surface layers of the fragments. At the University of Newcastle, S. K. Runcorn's group has shown that this iron is superparamagnetic and exhibits an inverse square law spectrum of grain volumes, extending to diameters much less than 13 nm.

The origin of this free iron, which is found in both mare and highland soils, is a topic of great current interest. It is non-meteoritic, being almost nickel free, and is thought to be produced by preferential sputtering by the solar wind. If so one might anticipate the presence of implanted carbon in association with it, and G. Eglinton's group at the University of Bristol has found just this. In place of the pyrolysis method, which determines only total carbon content, the Bristol group have developed an acid dissolution technique using deuterochloric acid, DCl, for their lunar sample studies. Trapped hydrocarbons release mainly CH_4 and may thus be distinguished from the acid-hydrolysable carbon, mainly the free element, which is released as CD_4. Eglinton *et al.* show that the content of hydrolysable carbon correlates strongly with iron content and magnetic susceptibility, although only for the Luna 20 samples did they note an increase in hydrolysable carbon with diminishing grain size. This may reflect the fact that Luna 16's basaltic soil is derived from a material of lower melting point than the gabbroic soil returned by Luna 20. The arguments used here are complex and will certainly be examined closely, though Eglinton's group has recently presented clear evidence for the intimate association of carbon and α-Fe in synthetic materials (Jull *et al. Nature* **262**, 566; 1976).

Like the Bristol workers, S. H. U. Bowie and colleagues (Institute of Geological Sciences) have produced very precise results from exceptionally small samples. Their measurements of oxygen isotopic ratios show an enrichment of ^{18}O (of about 0.65%), compared with terrestrial ocean water, in good agreement with previous measurements and apparently typical of the lunar regolith. This isotopic differentiation is also usually attributed to the sputtering action of the solar wind, so its value should relate to the exposure time of the material. Bowie's group find a complex variation of enrichment with grain size, and suggest that this may most reasonably be explained by the presence at one locality of impact ejecta from many distant sites, each with a different exposure history.

The last paper of the collection—by P. H. Cadogan and G. Turner (University of Sheffield)—describes the determination of ^{40}Ar–^{39}Ar ages of four samples, two each from Luna 16 and 20. The former yield an age of 3.4–3.5 Gyr for the Fecunditatis basalts, in excellent agreement with the Caltech group (*Earth planet. Sci. Lett.* **13**, 368 and 375; 1972). Apollo samples from the other major basins all show a range of ages of 0.2–0.3 Gyr, and the present lack of evidence for such an extended period in Fecunditatis cannot be regarded as significant on the basis of so few measurements. A metaclastic fragment from the Luna 20 sample, probably typical of the adjacent Crisium basin, yielded an age of 3.9 ± 0.1 Gyr.

Cadogan and Turner assign a minimum age of 4.3 ± 0.1 Gyr to their Luna 20 anorthosite fragments. There are now many highland samples dated to this period, and controversy has developed over how to interpret these ages. Are they evidence for a lunar cataclysm, comprising a few major basin-forming events, which ended 3.9 Gyr ago? Alternatively, can they be accounted for by a steadily decreasing cratering rate, resulting in a statistically-determined distribution in which individual ages reflect the latest event to reset the Ar clock? Cadogan and Turner favour the calaclysmic theory. Samples from Luna 24 should arrive at Houston in the spring and radiometric results from them are eagerly awaited. ☐

Morphogenesis of the mammary gland

from John K. Heath

UNDERSTANDING the mechanisms underlying the formation of organs poses a formidable problem to the developmental biologist. It is known that in most systems interactions between the epithelium of the organ rudiment and its adjacent mesenchyme are involved, but these interactions have proved to be complex. Two recent pieces of work which use the mouse mammary gland, present new experimental approaches which may provide an insight into this problem.

If the mammary epithelium is cultured in contact with mammary mesenchyme the tissues undergo typical mammary morphogenesis and differentiation, but if the epithelium is cultured alone its development is curtailed. If salivary gland is used as the source of mesenchyme, however, the subsequent morphogenesis of the mammary epithelium is typical of the salivary epithelium (Kratochwil *Devl Biol.* **20**, 46; 1969), but the biochemical properties of the mammary epithelium in this case, however, are similar to normal mammary glands *in situ*. Sakakura *et al.* (*Science* **194**, 1439; 1976) have now taken submandibular salivary gland mesenchyme from mouse embryos 14 days after conception and combined it with mammary epithelium from 16 day post-conception female embryos. After a brief period of culture the combined tissues were transferred to a site under the kidney capsule of adult female mice. Subsequent histological examination of the transplants revealed the typical salivary pattern of morphogenesis But if the mice carrying the grafts were mated and allowed to give birth and lactate, the ducts in the graft became distended with milk. Biochemical studies showed the transplanted tissues to be producing amounts of the B protein of lactose synthetase similar to those found in lactating mammary glands *in situ*. These findings show that although the mesenchyme plays an important part in determining the overall form, the cytodifferentiation of the gland is either already determined by 16 days or is independent of the underlying mesenchyme.

The use of heterologous mesenchyme in recombination experiments is common. But by the time experimental recombinations can be made many interactions involved in the formation of the organ may have occurred. Another problem is that although interactions can be demonstrated it is hard to tell which tissue takes the lead in determining the subsequent path of development. These difficulties have been circumvented by the exploitation of a mutant gene affecting the differentiation of the mammary gland (Kratochwil and Schwartz *Proc. natn. Acad. Sci. U.S.A.* **73**, 4041; 1976).

The mammary gland of the male can be distinguished from that of the female by the fourteenth day of gestation since it undergoes regression and either disappears completely or remains in a rudimentary state (depending on the strain of mouse used). The mesenchyme surrounds an epithelial bud and begins to condense around it. The bud then starts to undergo necrosis and the 'stalk' of the bud is cut off. This pattern of regression can be inhibited by destruction of the foetal testes and can be induced in females by administering testosterone. The involvement of exogenous substances such as hormones would seem only to complicate an already difficult situation. However Kratochwil and Schwartz have shown how the action of testosterone can be used as a starting point for unravelling the interactions involved in the process of regression.

They asked whether the hormone exerts its effect on the mesenchyme or the epithelium or both The key to the solution of this problem was the use of the X-linked mutant 'Testicular Feminisation' (Tfm), tissues of which are insensitive to testosterone (probably due to a malfunctioning hormone receptor molecule). Although a X^{Tfm}/Y mouse is genetically male it lacks male secondary sex characteristics, and in particular the mammary glands do not show the typical testosterone-induced regression. Kratochwil and Schwartz combined epithelium and mesenchyme from X^{Tfm}/Y and X/Y embryos and observed the effects of testosterone on their development. The regression response occurred only if the mesenchyme was wild type. This implies that testosterone initially acts on the mesenchyme and that the subsequent fate of the epithelium is determined by the mesenchyme. An interesting corollary of this observation is the implication that the necrosis of the epithelium is the result of killing by the mesenchyme as opposed to the cell-autonomous suicide typical of cell death in other systems.

The exploitation of appropriate mutants has proved of enormous value in other developmental problems and Kratochwil's work has opened up the possibility of their use in the study of organogenesis. In addition these experiments may lead to the use of other hormone-triggered tissue interactions for looking at epithelial/mesenchymal interactions. ☐

Hot white dwarfs and the EUV

from M. G. Edmunds

APART from the Sun, very few astronomical sources have yet been detected in the extreme ultraviolet (EUV) wavelength region 100–1,000 Å. The flight of the Apollo–Soyuz mission in July 1975 provided a platform for a grazing-incidence mirror telescope with a filter photometer, and the detection of two sources outside the Solar System has been reported. Both sources have good positional identification with white dwarf stars, and the second of these (Margon *et al. Astrophys. J. Lett.* **210**, 79, 1976) is Feige 24, a well-known spectrophotometric standard at visible wavelengths.

The EUV continuum observations have implied a considerably higher atmospheric temperature for these white dwarfs than had previously been assumed. At optical wavelengths the continuum radiation energy distribution is fairly insensitive to the atmosphere parameters, but model atmosphere calculations suggest that the fluxes in the EUV are more sensitive, especially shortward of the once-ionised helium absorption edge at 229 Å, provided that there is a significant helium abundance in the star's atmospheres. The temperature of the other white dwarf HZ43 has been inferred to be as high as 10^5 K, and it is detected as a very soft X-ray source Although high for a white dwarf, such a temperature is comparable with temperatures derived for the central stars of planetary nebulae by the Zanstra method—in which the ultraviolet flux from the star is indirectly estimated by observation of optical re-emission of the energy from the ultraviolet radiation absorbed by the nebular gas surrounding the star. Thus it is reasonable that HZ43 is a fairly young white dwarf, still very hot after passing through the planetary nebula stage, in which it shed its outer layers during its evolution from a giant.

The EUV observations and ultraviolet data from the Orbiting Astronomical Observatory 2 (Holm *Astrophys. J. Lett.* **210**, L87) imply that Feige 24 is not quite so hot, around 6 to 7×10^4 K. To predict the EUV fluxes, Margon *et al.* attempt a model atmosphere calculation for a pure hydrogen atmosphere, and they find that the low observed flux shortward of 170 Å implies that there must be at least some helium present in the atmosphere to provide enough opacity shortward of the ionised helium edge. They suggest a possible, but less probable, alternative in the presence of absorption by ionised helium in the interstellar gas. Feige 24 is known from spectroscopic evidence to be a binary pair of a white dwarf and a cool main sequence dwarf showing emission lines. By subtracting the expected infrared flux of the white dwarf from the total flux of the pair, Holm deduces the energy distribution and hence a rough classification for the main sequence star. By comparison with known main sequence stars, it is then possible to infer a distance of about 90 pc for the binary pair. For a spectral region shortward of the Lyman limit at 912 Å there is very strong absorption in the interstellar medium by bound–free transitions of neutral hydrogen. The detection of any EUV flux from Feige 24 at this distance indicates that the local interstellar hydrogen is only about 0.01 to 0.02 particles per cubic centimetre, very low compared with the mean value for the Galactic plane of about 0.6 particles per cubic centimetre. This reinforces the view that the interstellar medium is very clumpy, with strong concentrations into clouds coexisting with a tenuous intercloud medium.

It has been suggested that Feige 24 shows the spectral characteristics of old novae, in which accretion of gas from the red dwarf across onto the white dwarf has liberated radiation. If such processes are still occurring, the resulting radiation is liable to be variable, and the continued use of Feige 24 as a spectrophotometric standard might justifiably be questioned. ☐

Effects of photon momentum

from a Correspondent

THE photon dynamics resulting from light absorption by an atom are sufficient to cause an observable deflection of the excited atom. Radiation effects of this nature can now be utilised as techniques in atomic scattering experiments opening up a new and exciting area of investigation.

The advent of commercially available tunable continuous wave (CW) dye laser systems now enables atomic physicists selectively to prepare the target atom in a scattering experiment in a well defined excited atomic state. The tunable power currently available (several milliwatts at a bandwidth of 5 MHz) is sufficient to maintain the necessary excited state population required for a scattering experiment to be performed. The sodium atom with its resonance transition 3^2S–3^2P, suitably placed in the region of efficient organic dye operation is a very attractive candidate for optical pumping. Work on the scattering of state selected sodium atoms ($3^2P_{3/2}$) off neon atoms has been reported (Carter *et al.*, *Phys. Rev. Lett.* **35**, 1144; 1975) in the determination of ground and excited potentials of the NaNe molecules. Hertel *et al.* (*J. Phys. B.* **7**, 570; 1974) were the first to perform high resolution electron scattering experiments on laser-excited sodium atoms where elastic, inelastic and superelastic processes were studied.

In the work by Hertel only differential cross section $\sigma(\theta)$ measurements were made where the scattered electron was monitored over a limited angular scattering range θ. To establish a total cross section, measurement of $\sigma(\theta)$ is required over the angular range $\theta = 0$ to 180°. An alternative method for directly measuring the total cross section σ for electron atom scattering, that is, atomic beam recoil, was developed several years ago (Bederson in *Methods of Experimental Physics* (ed. Bedersen B. and Fite W. L.) **7A**, 89 (Academic, 1968) for measuring σf or the scattering of electrons off ground state atoms. In this procedure a well defined narrow atomic beam is cross fired at right angles by a beam of low energy electrons. The collimation in the apparatus after the interaction region is sufficient to enable the reduction in flux of the atomic beam to be monitored. The signal scattered out of the incident beam by the electrons $\Delta I = I_o - I_s$ (where I_o and I_s are the atomic beam current with the electron beam off and on respectively) therefore gives a direct measure of the total electron scattering cross section. The removal of atoms from the beam is the dynamical effect of the momentum transfer imparted by the scattered electron to the atoms and this is sufficient to cause atomic recoils of several degrees.

No scattering experiments so far have taken advantage of the dynamical effects of photon–atom interactions as a diagnostic method. The momentum transferred to an atom, of mass m and velocity V, by resonance absorptions, at wavelength λ, will result in a recoil through an angle α_o ($= h/m V\lambda$) with a recoil in the opposite direction for stimulated emission. Since spontaneous emission is random in direction the steady state recoil experienced by an atom passing through a laser beam will saturate at a value of $\alpha_o t / 2\tau$, where t is the time spent in the laser beam and τ is the natural life time of the atomic state being excited. In the experiment of Bhaskar *et al.* (*Phys. Rev. Lett.* **38**, 14; 1977) $\alpha_o \approx 3\times10^{-5}$ rad per photon absorption and the nett atomic beam deflection observed at 80 cm from the interaction region is several millimetres. This photon dynamical effect can now be combined with the atom recoil technique where the laser beam is perpendicular to the plane containing the atom and electron beams. This will yield the total cross section for scattering of electrons from the excited state $3^2P_{3/2}$ ($M_F = 3$) using the relation

$$\Delta I_{on} / \Delta I_{off} = f(\sigma_e / \sigma_g) - 1) + 1$$

where ΔI_{on} and ΔI_{off} are the atom scattering out signals with the laser beam on and off respectively, and σ_e and σ_g are the total cross sections for electron scattering off excited and ground state atoms respectively. Bhaskar *et al.* have measured the absolute value of σ_e at an incident electron energy of 4.4 eV and obtained a value of $(285 \pm 55)\times10^{-16}$ cm^2.

Extension of this technique to differential cross-section measurements and to experiments in which the spin state of the atom is selected will greatly increase the knowledge of fundamental processes in atomic scattering. ☐

A hundred years ago

A MEDAL to commemorate the part taken by the Institute of France in the observation of the transit of Venus has been struck at the national mint. It bears the representation of a female passing before the car of Apollo, with the motto in Latin, "Quo distent spatio, sidera juncta docent." Each member of the Institute has received a silver medal, as well as the heads of the mission; the assistants received a bronze one. A medal has been cast in gold and presented to M. Dumas, the President of the Transit Commission. The expenses were defrayed by subscription among the members of the Institute.

From *Nature* **15**, February 15, 344; 1877.

review article

Strategy for detection of cancer hazards to man†

Sir Richard Doll*

Forty-four years ago, Sir Ernest Kennaway and his colleagues identified, for the first time, a pure chemical that was capable of causing cancer in animals, and a year later isolated another from material that was widespread in the environment. At that time, and even after these crucial observations, it was commonly assumed that cancers were an inevitable accompaniment of ageing and that little could be done to reduce the mortality they caused. It is now clear, however, that most, if not all cancers have environmental causes and can in principle be prevented. The identification of environmental hazards and clarification of the mechanisms through which they cause disease are thus among the highest priorities in cancer research.

Since Kennaway's seminal work[1,2], the evidence that cancer can be prevented has accumulated steadily and is now overwhelming. Whether it will ever be possible to prevent the disease altogether, as we can now prevent poliomyelitis and scurvy, is impossible to say until we know more about the mechanism by which it is produced; but we should be able to reduce the age-specific incidence rates—which account for a quarter of all deaths of men in Britain under 75 years of age—by at least 80 to 90%. That this is so, is suggested primarily by the great variation in the incidence of different types of cancer in different communities and in different parts of the world.

This variation is illustrated in Tables 1 and 2, which show maximum and minimum incidence rates for all cancers that are common enough somewhere for the disease to affect more than 1% of men or women by 75 yr, in the absence of other causes of death. The range of variation is never less than fourfold and is sometimes more than a hundredfold. I have presented the data as cumulative incidence rates up to 75 yr, despite the fact that rates at old ages tend to be unreliable where personal medical services are sparse, to illustrate how common some cancers can become in countries like our own where half the population lives to be over that age. In estimating the range of variation, however, I have limited comparison to ages under 65 yr.

With rare cancers, the very fact of their rarity makes the demonstration of their incidence in the small populations that have been studied outside the industrialised countries extremely difficult, if not impossible. Nevertheless, some clearly vary; for example, Burkitt's lymphoma, which never affects more than 1 in 1,000 people[3], varies at least 100-fold. Others, such as acute leukaemia in young adults and nephroblastoma in children, seem to be fairly constant everywhere.

Some of this variation is, of course, genetic in origin. That heredity is not the principal cause is, however, shown by migrant groups, whose experience of disease usually changes when they change their way of life in a new country, and by the variation in the incidence of cancer with time. The latter can seldom be demonstrated conclusively, partly because it is difficult to compare the efficiency of case finding at different periods, and partly because a high standard of case registration has obtained in only a few places for more than 10 yr—the most notable being Denmark and Connecticut, USA. We have, therefore, to rely to a large extent on trends in mortality, which may also be influenced by changes in the efficacy of treatment.

Nevertheless, some changes are so gross that it is impossible to doubt that there has been a real change in incidence: for example, the increase in lung cancer in all developed countries, the increase in oesophageal cancer in the black population of South Africa, and the decrease in gastric cancer in the USA. Many lesser changes have taken place in Britain during the last 20 years. These are shown in Table 3, which lists all those cancers for which the mortality rate, standardised for age, has changed by more than one per cent a year between 1958 and 1973. Several of these changes cannot be dismissed as artefacts, because the disease is easy to diagnose and the death rate has increased despite improving treatment (as with melanoma and cancer of the testis). Taken altogether, the evidence suggests that all common cancers have varied in incidence from time to time, just as they now vary from place to place.

Much of this variation can now be attributed to the action of specific agents. Indeed, so many are now known that we may reasonably hope to extrapolate from past experience in seeking others. I shall, therefore, begin by reviewing briefly the known agents and the ways in which they have been detected.

Iatrogenic hazards

About a score have been prescribed by doctors (Table 4). Most have caused only a few cases and their effect has been apparent to clinicians and pathologists because the microscopic appearance of the tumour or the site in which it occurred was so unusual. Examples include adenocarcinoma of the vagina in young women whose mothers had taken stilboestrol during the relevant pregnancy (recognised when a lift broke down in Boston and the paths of a gynaecologist and a pathologist crossed for sufficiently long for them to exchange experiences); angiosarcoma of the liver and spleen following the injection of thorotrast; squamous carcinoma of the thigh following the local application of coal tar ointment; squamous carcinoma of the

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†This article is based on the Sir Ernest Kennaway Lecture delivered at the Royal Institution on 11 November 1976.

Table 1 Range of variation in the incidence of common cancers in men (unless specified ♀)

Type of cancer	High incidence area	Cumulative risk by 75 years of age* (%)	Range of variation†	Low incidence area
Skin	Australia, Queensland	>20	>200	India, Bombay
Oesophagus	Iran, NE	20	300	Nigeria
Bronchus	England	11	35	Nigeria
Stomach	Japan	11	25	Uganda
Cervix uteri ♀	Colombia	10	15	Israel (Jewish)
Liver	Mozambique	8	70	Norway
Prostate	USA (black)	7	30	Japan
Breast ♀	USA, Connecticut	7	5	Uganda

*In absence of other causes of death.
†At ages 35–64 years.

palm of the hands associated with arsenicism; reticulosarcoma of the brain following immunosuppression to prevent rejection of a renal transplant; and a peculiar type of liver tumour in women using steroid contraceptives.

The only known agents in this group that have caused any substantial number of cancers have been ionising radiations, which probably caused between 5 and 10% of all childhood cancers in Western Europe and North America during the 1950s and 60s, when used for diagnosis during the pregnancy of the mother; and oestrogens, prescribed for post-menopause women, which may account for much of the recent increase in the incidence of endometrial cancer in the USA. In both cases, the risks were overlooked for a long time, because the tumours did not differ in any obvious way from the tumours that occurred quite commonly as a result of other causes, and they were eventually detected only after large scale case-control studies had been carried out with the object of investigating the role of the agents in the aetiology of the particular disease[1-4].

Despite the number of tumours caused by these last two agents, the total number of cases that are iatrogenic in origin is much less than readers of Illich's *Medical Nemesis* might suspect and cannot have constituted more than a minute proportion of the total impact of cancer on society.

Occupational hazards

Other agents have caused hazards in a wide variety of occupations. These are listed in Tables 5 and 6. In three instances the risks were discovered after the agents (4-aminobiphenyl, mustard gas and vinyl chloride) had been shown to cause cancer in animals. Other agents were discovered incidentally in the course of investigating other industrial hazards, as in the case of cancer of the prostate in cadmium workers and cancer of the lung in rubber workers. Most, however, were discovered because the interest of a clinician, an epidemiologist, or a pathologist was aroused by the observation of a cluster of cases that seemed too large to be easily attributable to chance. Sometimes these clusters have been quite small, as when Dr John Jones reported that he was disquieted to have seen two employees of the Mond Nickel Company develop nasal sinus cancer within a year. Often, however, the risk has been overlooked for a long time, particularly when the same type of cancer was common in the general population as a result of other causes.

Like the iatrogenic hazards, these occupational ones cannot have been responsible for more than a very small proportion of all cancers, as the total number of men who have been exposed in the course of their work, other than open air workers exposed to ultraviolet light and, to a lesser extent, the wide variety of workers exposed to asbestos, is small in proportion to the population as a whole. They are important, however, for two reasons.

First, they are important to the workers concerned who have had a 50% risk of developing the disease in some industries. Indeed, in one small group of 19 men employed on distilling 2-naphthylamine, the risk proved to be 100%, 18 dying of bladder cancer and the last having been killed in an accident shortly after the disease was diagnosed. Second, some of the agents concerned have found their way into the general environment, so that millions of people have been exposed to them unintentionally and sometimes in an uncontrolled way. These agents are listed in Table 6. It is easy enough to dismiss the corresponding risks on the grounds that the doses are minute; but we can no longer assume that thresholds exist for chemical or physical agents and we ought neither to ignore nor to condemn them until we have derived quantitative relationships between the dose to which individuals are exposed and the resultant incidence of the disease. At present we can do this only very crudely. Nevertheless, any quantitative evidence is better than none.

Industrial pollution

Consider, for example, polycyclic hydrocarbons and asbestos. For the first, we have evidence that the large amount of benzo(*a*)pyrene inspired by gas retort house workers produced a risk of lung cancer only about 80%

Table 2 Range of variation in the incidence of common cancers in men (unless specified ♀)

Type of cancer	High incidence area	Cumulative risk by 75 years of age* (%)	Range of variation†	Low incidence area
Colon	USA, Connecticut	3	10	Nigeria
Buccal cavity	India, part	>2	>25	Denmark
Rectum	Denmark	2	20	Nigeria
Bladder	USA, Connecticut	2	4	Japan
Ovary ♀	Denmark	2	8	Japan
Corpus uteri ♀	USA, Connecticut	2	10	Japan
Nasopharynx	Singapore (Chinese)	2	40	England
Pancreas	New Zealand (Maori)	2	5	Uganda
Penis	Uganda, part	1	300	Israel (Jewish)

*In absence of other causes of death.
†At ages 35–64 yr.

greater than the national average[8]. Since the residents of large towns are unlikely to have been exposed to more than one-hundredth of that amount—mainly from the combustion of domestic coal[9]—the contribution of these agents to the urban excess of the disease is unlikely to have been large, a conclusion that is confirmed by the low incidence of the disease in non-smokers irrespective of where they live.

As for asbestos, we know that it reaches the ambient air from a variety of sources, including the clothes of asbestos workers. These were presumably the principal source of exposure of the 37 men and women in nine countries who are reported to have developed mesothelioma of the pleura after being household contacts of asbestos workers[10]. The maximum concentration that has been found in the air near building sites where asbestos was being sprayed is, however, three orders of magnitude less than that which has been regarded as an acceptable concentration in the asbestos industry[11], and the amount that is commonly present in town air is still less by another two orders of magnitude. Unfortunately, we are still uncertain about the size of the risk that is associated with this accepted concentration of about 0.1 mg m^{-3}. In one study of an asbestos textile factory in England[12], it was found that the relative risk of lung cancer for men who had been employed for 20 yr decreased progressively from 10 times 'normal', if they had been employed for at least 10 years before 1933 (that is, the date when some control of asbestos dust in the air of factories first became effective in the UK), to 3½ times normal if they had been employed before 1933, but for less than 10 years, to 1½ times normal if they had first been employed only after 1933. Few men who were first employed only after 1951 have yet been employed for 20 yr, but preliminary estimates (which are highly unreliable because the number of men observed is so small) suggest that some risk may still have persisted since that date. Detailed dust records were not obtained in the early years in a way that enables them to be compared with present data, but the amount of dust in the air was initially gross and continued to be much greater than at present for some time after 1933. Even in 1951, the mean dust level to which men were exposed is likely to have been 3½ times greater than it is now, and we shall have to wait for many years before industrial data enable us to make a reasonable estimate of the possible risk associated with an average exposure up to 0.1 mg m^{-3} during normal working life.

Table 3 Changing mortality rates from different cancers* (England and Wales, 1958 to 1973)

Type of cancer	(%) Change	
	Males	Females
Melanoma	+95	+47
Myelomatosis	+65	+70
Lung	+29	+94
Pancreas	+19	+20
Testis	+19	
Oesophagus	+16	+17
Stomach	−25	−34
Buccal cavity	−38	−24
Cervix		−23

*All cancers with rate of change equal to or more than 1 % per year.

Measurements like these exculpate individually as major contributors to the gross variation in cancer incidence between countries all the agents that are known to cause specific occupational hazards (other than ultraviolet light), whether acting within industry or as pollutants in the general environment. In so far as we can explain this variation, it seems rather to be due to differences in social behaviour, diet and the opportunity for infection.

Table 4 Iatrogenic causes of cancer

Agent	Site of cancer
Diagnostic or therapeutic X rays	All sites
Thorium	Bone
Thorotrast	Liver, spleen marrow (leukaemia)
Polycyclic hydrocarbons in coal tar ointments	Skin
Alkylating agents	
Melphalan, cyclophosphamide	Marrow (leukaemia)
Oestrogens	Endometrium, breast (M) ? breast (F)
Stilboestrol (transplacental)	Vagina
Steroid contraceptives	Liver
Androgens (anabolic steroids)	Liver
Arsenic	Skin, lung
Chlornaphazine	Bladder
Immunosuppressive drugs	Reticulosarcoma
Phenacetin	?Renal pelvis

Other hazards

The other agents that are known to cause cancer are listed in Table 7. The number is small, but the cancers that have been produced are legion. Cancers of the buccal cavity attributable to chewing, for example, account for a quarter of all cancers in men in parts of India, while cancers of the lung attributable to smoking account for more than a third of all lethal cancers in men in Britain.

One agent, aflatoxin, was discovered to be a powerful carcinogen as a result of an outbreak of poisoning that killed 100,000 ducks and turkeys in British farms. The outbreak was traced to a consignment of peanut meal contaminated with *Aspergillus flavus* which produced a metabolite that not only caused acute liver failure in poultry, but also caused cancer of the liver in minute doses in a wide variety of animals[13,14]. Human liver cells contain the enzymes necessary to produce the active agents (the epoxy-metabolites of aflatoxin), and *Aspergillus flavus* is a frequent contaminant of foodstuffs stored under hot and humid conditions. Now it has been shown that the incidence of liver cancer in Thailand, Singapore, Kenya, Swaziland and Mozambique is proportional to the amount of aflatoxin in the diet[15,16]. The vagaries of daily diet make it unlikely that we shall ever be able to establish the relationship in individuals; but the total evidence is strong enough to justify an attempt to prevent the disease by reducing exposure. Unfortunately, this will not be easy, as fungal contamination is difficult to avoid under the conditions in which the staple foods are commonly stored in the tropics, and it will be expensive to substitute alternative methods.

The other agents listed in Table 7 were, for the most part, discovered as a result of clinical acumen dating back

Table 5 Occupational causes of cancer not contributing to general environmental pollution

Agent	Site of cancer
Ultraviolet light	Skin
Aromatic amines	Bladder
2-naphthylamine	
1-naphthylamine	
Benzidine	
4-aminobiphenyl	
bis-chloromethyl ether	Bronchus
Benzene	Marrow (leukaemia)
Mustard gas	Bronchus Larynx Nasal sinuses
(Nickel ore)	Bronchus Nasal sinuses
(Chrome ore)	Bronchus
Cadmium (?)	Prostate
Agents in isopropyl oil hardwood furniture manufacture leather goods manufacture	Nasal sinuses

Table 6 Occupational causes of cancer contributing to general environmental pollution

Agent	Site of cancer
Ionizing radiations	Bronchus Skin Bone Marrow (leukaemia)
Polycyclic hydrocarbons in soot, tar, and oil	Skin, scrotum Bronchus
Arsenic	Skin Bronchus
Asbestos	Bronchus Pleura, peritoneum
Vinyl chloride	Liver (angiosarcoma)

nearly 200 years to Soemmerung's description of lip cancers in pipe smokers[17]. Clinical associations, however, tend to be overlooked if the conclusions suggested are socially unacceptable, and little attention was paid to the possible effects of smoking until the death rate from lung cancer in men had increased 25-fold (Fig. 1). The conference that was called by the Medical Research Council in 1947 could not decide whether the increase was real or an artefact due to improved diagnosis, but it did recommend a study to seek a possible cause. The subsequent enquiry showed that patients with lung cancer tended to smoke more than other patients, and within 3 years it was possible to conclude from the human evidence alone that cigarette smoking was a cause of the disease[18]. Whether these observations, or any others that are both practicable and ethical to obtain, can be regarded as constituting logical proof that a carcinogenic agent has been detected is debatable and is not of great interest. What is of interest is whether the evidence justifies an attempt at prevention and, if so, whether removal of the agent is followed by the result we seek[19].

Prevention should, of course, ideally be carried out in a controlled way with random allocation of individuals, or groups of individuals, to experimental and control series. In practice, however, this is seldom possible as the experiment is likely to require the cooperation of a large number of people who have to be convinced that it will be successful before it can be begun. Everyone, therefore, will want to be in the experimental group. This is what has always happened in industry; but when the disease disappears, as so many occupational cancers have, few people have challenged the logic that led to the intervention.

Once smoking began to be studied seriously as a cause of cancer, data were soon collected that confirmed the old clinical observation of a relationship with cancer of the mouth, and there are now strong grounds for believing that it also causes many cancers of the pharynx, larynx and oesophagus. Weaker, but consistent, evidence—like that summarised in Table 8—also suggests that it may play a part in producing cancers of the bladder and pancreas.

That alcohol contributes to the causation of a large proportion of cases of cancer of the oesophagus, and to a somewhat smaller proportion of cancers of the mouth, pharynx and larynx, in some countries, has been suspected ever since the mortality from these cancers was found to be unusually high in publicans, waiters and others employed in alcoholic trades[20]. The subject has, however, proved so unattractive to research workers that we still do not even know whether it is the alcohol itself that is responsible (possibly by solubilising a specific agent) or whether it is another component of alcoholic drinks that may vary in amount from one drink to another. That the subject has been unpopular may have been because pure alcohol is not carcinogenic to laboratory animals or because the amount consumed by the individual is difficult to assess with accuracy—or even because of a natural aversion for investigating such an unpopular subject. Interest in it has, however, been revived and studies are now being carried out on oesophageal cancer in parts of France where the characteristic alcoholic drinks are cider and cider-based liqueurs[21]. If, however, alcoholic drinks are important in the aetiology of cancers of the upper digestive tract in Europe and North America, they certainly do not explain the astronomical figures for the incidence of the disease in central Asia, where very little alcohol is consumed.

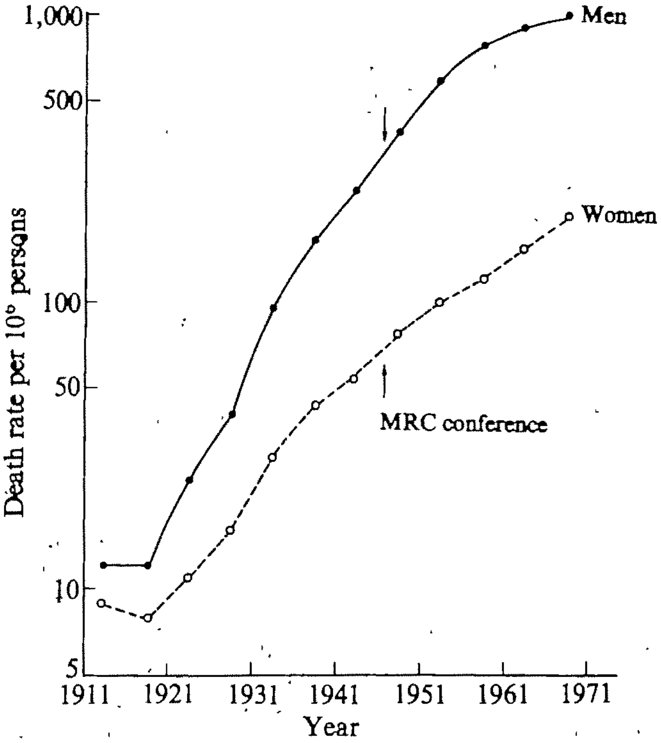


Fig. 1 Trend in crude death rate from lung cancer 1911–1971, by sex, showing state at time of MRC conference.

Viruses

Another potential hazard is infection with an oncogenic virus, but it is still an open question whether viruses can ever cause cancer in man. Direct evidence of case-to-case infection is extremely weak—no-one, for example, who has used adequate controls has been able to repeat Vianna, Greenwald and Davies's[22] observation that patients with Hodgkin's disease have had unusually close personal contact—and the laboratory evidence that cancers are associated with viral infection is open to several interpretations.

Table 7 Other environmental causes of cancer

'Agent'	Site of cancer
Sunlight	Exposed skin (rodent ulcer, squamous carcinoma, ? melanoma)
Associated with use of 'Kangri' and 'dhoti'	Skin of abdomen, groin, and thigh (squamous carcinoma)
'Reverse smoking'	Palate
Chewing betel, tobacco, lime	Mouth
Smoking	Mouth, pharynx, larynx, bronchus, oesophagus, bladder, ? pancreas
Aflatoxin	Liver
Schistosomiasis	Bladder
Associated with sexual intercourse	Cervix uteri

Table 8 Risk of death from cancer of pancreas in continuing cigarette smokers relative to life-long non-smokers (prospective studies)

Population*	Sex	Risk compared with that in non-smokers: Cigarette smokers	Current cigarette smokers smoking per day: 1–9	10–20	21–39	40 or more
US citizens						
(77)	F	1.8				
(108)	M	2.7				
US veterans						
(493)	M		0.9	1.9	2.2	1.9
Canadian pensioners						
(89)	M		1.4	2.0	2.4	
Swedish random sample						
(46)	M		1.6	3.4	5.9	
British doctors						
(92)	M		1.4	1.4	2.1	

*Numbers of deaths in parentheses: for details see Hammond[20], Kahn[21], Department of National Health and Welfare[22], Cederlof *et al.*[23] and Doll and Peto[19].

Consider, for example, the extensive evidence that now connects the EB virus with Burkitt's lymphoma and carcinoma of the nasopharynx. In epidemic areas these diseases occur nearly always in individuals infected by the virus. Viral DNA is present in all the tumour cells and determines the expression in them of virus-coded neoantigens; and virus production can be activated in some of the tumour cells in the laboratory. The EB virus is widespread in human society and is the cause of infectious mononucleosis, when it stimulates the proliferation of mononuclear cells of the lymphatic series. *In vitro*, it confers the property of continuous growth on normal human B lymphocytes in culture, in a way that is analogous to malignant transformation. Finally, it has been shown to cause malignant lymphomas in South American cotton top marmosets on experimental inoculation.

What more can laboratory investigation be expected to do? The International Agency for Research on Cancer[26] is attempting to relate the development of the disease to new infection by following up children in the West Nile district of Uganda from whom blood samples have been taken for serum studies; but the results are unlikely to be decisive if, as one suspects, viral infection is only one of several conditions necessary for the development of the disease. If this turns out to be so, the only remaining approach will be to develop a vaccine that can be shown to prevent the disease[27] —immensely difficult though that must be—and the same would also be true if cervix cancer were firmly linked to infection with the type II herpes simplex virus or to any other infective agent.

That cervix cancer is venereal in origin is now virtually certain. We know that the disease spares nuns and is most common in prostitutes; that the risk increases with the number of marriages and with the age at which coitus first occurs, but not with the number of pregnancies, nor with the frequency of intercourse within marriage; and that more of the husbands of affected women have had extramarital intercourse than of the husbands of control women[28–30]. To these facts we can add Beral's observation[31] that the mortality from cervix cancer in cohorts of women of different ages varies with the incidence of gonorrhoea at the time they were 20 years old, and the accumulating evidence that obstructive methods of contraception are protective[32].

Interaction of agents

If viruses do, in fact, cause cancer, they may do so only by interacting with other factors, and this may account for the separate correlation of hepatitis B antigen and aflatoxin with hepatoma and of the EB virus and gross malarial infection with Burkitt's lymphoma. That two different agents may interact to produce cancer has been established by occupational studies which have demonstrated interactions between smoking and asbestos and smoking and ionising radiations and have provided quantitative data like those on the incidence of lung cancer in uranium miners in the USA[33].

Similar data are now also being obtained by the International Agency for Research on Cancer[26] for the interaction between tobacco and alcohol in the production of oesophageal cancer in France. Preliminary estimates, based on a retrospective case-control study of patients and a random sample of the population, suggest that the risk of developing the disease increases both with the amount smoked and with the amount drunk, until among men who drink 81 or more grams of ethyl alcohol (equivalent to 7 whiskies) and smoke 20 or more cigarettes a day, the risk is 45 times that in those continent men who drink less than 40 grams and smoke less than 10 cigarettes a day.

The existence of such interactions, which has been suspected since the early experiments of Rous and Kidd[34] and Berenblum and Shubik[35], can be of great importance. For they may provide not only the explanations for apparently conflicting observations, but also alternative means of preventing disease, one of which may be more practicable than the other. If, for example, smoking and asbestos interact to produce bronchial cancer, there may be no point in trying to give quantitative labels to their respective shares. Both may be responsible for producing more than half the cases in the sense that the elimination of either would reduce the total risk by more than 50%. The important conclusion is that we can break the chain of causation at either of two links, and it may be as idle to try and partition responsibilities as it is to try to quantify the relative contributions of nature and nurture to disease in general.

Strategy for detection

In this incomplete review, I have sought to provide an account of the hazards of cancer to man and to use the experience of the past to indicate how other hazards can be detected in the future. We cannot, of course, hope to detect hazards efficiently until we know how cancer is produced, so that a policy for detection must include the support of basic biological research. Success in this field is dependent on the development of ideas and is difficult to foster except by providing the conditions in which outstanding investigators are able to give full rein to their imagination.

Test of products before use

Two types of hazard stand out as socially unacceptable, although they are relatively unimportant numerically: that is, the hazards associated with occupation and the use of prescribed drugs. The evidence that chemical carcinogens, or their reactive metabolites, are commonly electrophilic, react with DNA and, in consequence, are able to induce mutations, has suggested that it may be possible to predict carcinogenicity by relatively quick and simple tests with bacteria. It is not certain that any one test, or combination of tests, will prove to be adequate, but preliminary results are encouraging[36].

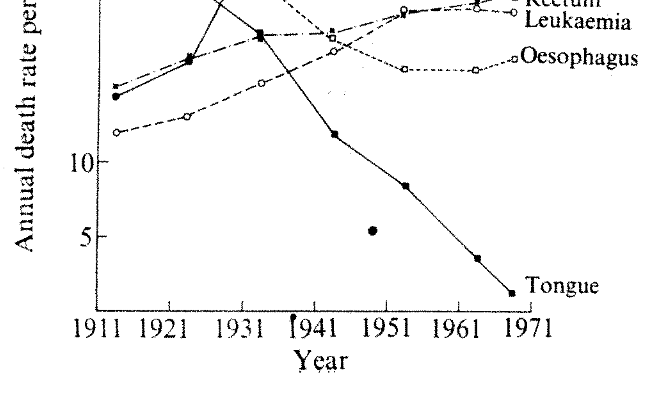
This, however, will not absolve us from making arrangements to detect hazards to man. Not only must we expect some chemicals to escape the net, particularly if they are hormones or act as promoters, enzyme inducers, or repair inhibitors; but we shall also wish to use some, and possibly many, substances that give positive results in animals, if the benefits are judged to be great enough to outweigh the harm. The continuing challenge to laboratory scientists is to discover enough about metabolism and the mechanisms of carcinogenesis in man to enable us to predict the extent of the hazard if the substance is used in a particular way. Meanwhile, we must either refrain from introducing such immensely useful materials as phenobarbitone, isoniazid, DDT, saccharin, and the minute amounts of stilboestrol that are used to fatten cattle, although they do not have any detectable effect in man, or arrange to detect and quantify hazards to man at the earliest opportunity.

Record linkage

Embryo mechanisms suitable for this purpose already exist in Britain, that are based on the records of the National Health Service and the Office of Population Censuses and Surveys, and to a varying extent in other countries[37–40]. Identifying information about all people who develop cancer is already integrated into a single file in England and Wales, and we now need to create similar files of people who are exposed to different chemicals. The Registrar-General has begun to do this by maintaining a file of a 1% sample of the population which is up-dated at each successive census in respect of births, deaths, immigrants and emigrants, and by recording the cumulative occupational experience of the selected sample throughout their lives. This file can be linked within OPCS to the occurrence of cancer and so can provide an automatic assessment of the risks associated with any particular industry. It will be necessary, however, to increase the size of the sample to at least 10% before it will be of any value for any but the larger risks. Very little information is included about specific exposures and it will probably also be necessary to require industry to maintain records of all employees who are exposed to new chemicals, so that they can be checked periodically against the file of cancer cases. Similarly, if we are serious in our desire to control adverse reactions to drugs, including reactions like cancer which occur only infrequently or after a prolonged latent period, we shall have to introduce some system for recording drug usage such as Skegg[40] is now doing on a pilot scale in 20 practices, or possibly create a new category of drugs that can be prescribed only on special forms for a period after they are introduced. Data that identify the patients can then be stored in a computer for subsequent matching with similar data in the cancer index.

The concept of linking records by computer has given rise to anxiety lest they are used to the detriment of the individual, as has happened when personal records have been linked by credit organisations in the USA, and some doctors have been worried by the apparent loss of confidentiality. Confidentiality can, however, be protected in a computer much more easily than in the standard case-note, and I know of no instance where the provision of personal information to a bona fide medical research worker has been abused. It is, of course, for the public to decide; but in my experience, most people understand that we cannot protect them against disease unless we are allowed the necessary tools. Record linkage of the type required is, moreover, not expensive when the essential records have to be made for other purposes, as they now all are—the only defect of the present system being that the records are not organised in a way that enables them to be used to detect hazards to health.

Fig. 2 Trends in mortality from common cancers in men 1911–1971, standardised for age.



Cancer incidence and mortality rates

Record linkage is less useful as a means of detecting hazards arising from environmental pollution or household materials, if there is no greater human exposure at the source of their creation or manufacture. In these circumstances we can rely only on cancer and death registries to arouse suspicion by revealing changes in the incidence of disease with time or place. Such registries are, also, one of our principal weapons for obtaining clues to other hazards that are not iatrogenic, occupational or attributable to pollution and which account for the mass of cancers that fill our hospitals today. Death rates for most of these and other cancers that were common between 1911 and 1970 are shown in Figs 2 and 3. The rates have been standardised for age and are limited to ages under 65 years, as diagnostic services for old people were so much less effective in the earlier part of the period. All the trends must have been affected by changing diagnostic standards and many will also have been affected by improving treatment, but we cannot avoid the conclusion, which accords with clinical experience, that cancers of the stomach, colon and rectum, and cancers of the breast and uterus (though not of the ovaries) have been common for many years so that if they are environmental in origin, as the evidence of geographical and social variation suggests, the responsible factors must have been prevalent at least before the beginning of the century.

Investigation of diet

One feature of the British way of life that has distinguished it from the Asiatic or African way for the appropriate length of time is its diet, and factors related to diet have been suspected of contributing to the production of cancer for many years. Until recently, however, there has been little precise evidence and very few promising ideas. Now, a spate of fresh ideas enables dietary factors to compete for the interest of research workers on almost equal terms with

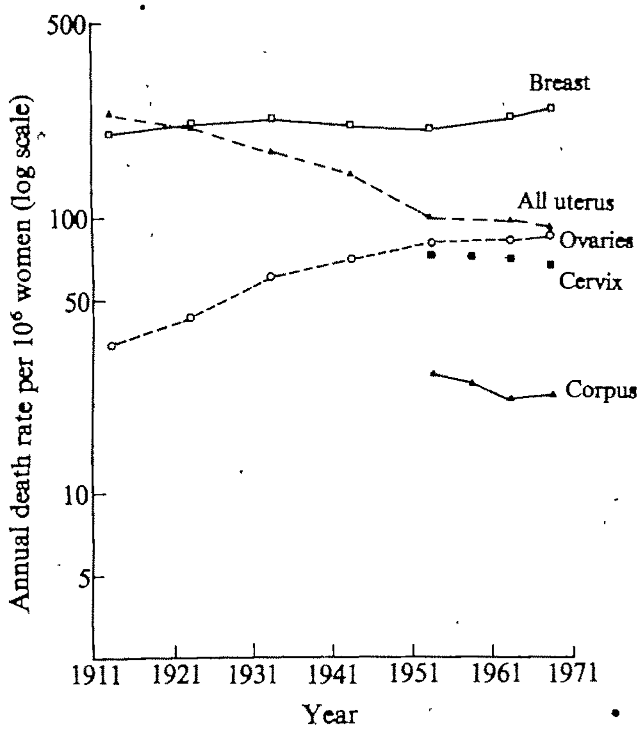


Fig. 3 Trends in mortality from common cancers of breast and genital tract in women, 1911–1971, standardised for age.

viruses and immune reactions. One such idea, to which I referred previously, was that aflatoxin might be responsible for tropical liver cancer. Another was the discovery that the cycad nut contained a constituent (cycasin) that was harmless in itself, but which gave rise to a carcinogen in the bowel of an animal with a normal intestinal flora. This was not fruitful in itself, but it focused interest on the possible role of intestinal bacteria and led Williams to examine the intestinal flora of people living in areas where the incidence rates of colon cancer were grossly different. Populations with a high risk were found to be characterised by the presence in their faeces of large numbers of anaerobic bacteria that were able to dehydrogenate bile acids, and Williams and his colleagues postulated that this might result in the production of carcinogens that acted locally on the colonic mucosa[41]. In 1975, Meade and his colleagues at Northwick Park, working in conjunction with Williams' group, reported the preliminary results of a retrospective study of patients with and without large bowel cancer which supported this idea[42], and these findings have now been confirmed in twice as many patients[43]. The original hypothesis will, however, have to be elaborated if it is to account for the low incidence of the disease in Finland and the increased incidence in the upper socio-economic classes in Hong Kong[43,44]; and Hill[43] suggests that it may also be necessary to have a high level of vitamin K to act as a hydrogen acceptor, before carcinogens can be formed under anaerobic conditions in the gut.

Burkitt's[45] hypothesis, that the relative lack of indigestible fibre in the diet of industrialised countries might be the primary cause of a variety of diseases of the large bowel that are absent in populations whose diet consists of natural unprocessed foods has also opened up new lines of thought. The greater bulk of faeces and the more rapid transit time associated with a high fibre diet could hardly account in physicochemical terms for the sort of gross differences in the incidence of large bowel cancer that occur between black African and the English-speaking countries; but the altered conditions in the bowel might perhaps account for the variation in the intestinal flora.

A fourth way in which diet may affect the incidence of cancer is by providing the raw materials from which nitrosamines are produced in the stomach—a group of powerful carcinogens that are still looking for human cancers to induce. Potential carcinogens of this group can be formed in acid gastric juice by the action of nitrites on secondary amines in food—the nitrites being present because of their use as preservatives, or because they were formed from nitrates in the mouth as a result of bacterial metabolism. This, however, is speculative. All we know for certain is that gastric cancer was common in the relatively poor populations of northern Europe and Japan (though not in most of the even poorer populations of Africa), that it has been becoming progressively less common in Western Europe and much less common in the USA, that it is less common in Japanese migrants to California and Hawaii than in the Japanese in Japan, and that the disease in the migrants tends to occur in those who eat pickled vegetables and dried and salted fish and not in those who eat such Western vegetables as tomatoes, celery, corn, lettuce and onion[46]. Could this be, as Weisburger and Wynder[47] suggest, because the small amounts of vitamin C inhibit the formation of nitrosamines in the stomach? Alternatively, it may be that the progressive reduction in incidence is due to better preservation of food, with a consequent reduction in the opportunity for bacterial or fungal contamination.

That vitamins may play a part in protecting against cancer is also suggested by experiments[48] on animals in which the incidence of cancer was reduced by a vitamin A-enriched diet. Now two sets of human data—one a prospective study of men with known dietary histories[49] and another a biochemical investigation of serum A levels in patients and controls[50], suggest that a deficiency of vitamin A may increase the risk of squamous cell carcinoma of the bronchus in cigarette smokers.

Fat also is a possible factor. Its consumption is closely correlated with the incidence of colon cancer ($r=0.78$) and breast cancer ($r=0.79$) in different countries, and several investigators have suggested that its content in the diet may directly affect the incidence of these diseases[51,52]. Armstrong[53] recently pointed out that the incidence of endometrial cancer was even more closely correlated with dietary fat ($r=0.85$) and showed that excess consumption of fat could account for five of the clinical characteristics that tend to be associated with the development of this disease; that is, obesity, early menarche, late menopause, maturity onset diabetes, and hypertension. Virtually all oestrogen produced in post-menopausal women is derived from oestrone which, in turn, is produced by aromatisation of the androstenedione secreted by the adrenals. Fat could play a part either because androstenedione is converted to oestrone in adipose tissue[54] or, perhaps, by inducing the oxidase systems that metabolise pre-carcinogens.

Conclusion

I have concluded by referring to some of the hazards that may be associated with diet, not because I believe that their existence has been proved, but because they provide an indication of the way in which the hazards that are responsible for the majority of human cancers may be detected in the future; that is, by a combination of epidemiological and laboratory enquiries. Such studies were often conducted independently in the past, with very little contact between the research workers involved. The position was, however, transformed when the World Health Organization set up the International Agency for Research on Cancer with the explicit purpose of encouraging such collaboration internationally, and it has been further strengthened in this country now that the Cancer Research Campaign, the Imperial Cancer Research Fund and the Department of Health and Social Security have joined the Medical Research Council in establishing academic units of cancer epidemiology within, or in close association with, the major departments of basic cancer research. With this collaboration, a rational use of medical records, and continued support for general biological research, I see no reason why causes for the remaining common cancers should not be detected within one or two decades. That is not to say that it will be easy to prevent the disease. For if, as I suspect, these hazards are associated with the common diet of developed countries, the problems that we are now having to face in preventing tobacco-induced cancer will seem childishly simple.

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articles

Rapidly-formed ferromanganese deposit from the eastern Pacific Hess Deep

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A thick ferromanganese deposit encrusting fresh basaltic glass has been dredged from the Hess Deep in the eastern Pacific. Contiguous layers within the Fe–Mn crust have been analysed for uranium-series isotopes and metal contents. The rate of accumulation of the deposit, based on the decline of uranium-unsupported ^{230}Th, is calculated to be approximately 50 mm per 10^6 yr. Based on hydration-rind dating of the underlying glass and an 'exposure age' calculation, this rate is concluded to be too slow, and an accretion rate on the order of 1 mm per 10^3 yr is more consistent with our data.

INDURATED metal-rich crusts associated with probable hydrothermal areas on the seafloor have been reported[1–4]. Ferromanganese encrustations, sampled on active spreading centres, include those from the Mid-Atlantic Ridge, sampled during the Trans-Atlantic Geotraverse (TAG) expedition[2,3] and during the FAMOUS project[4], from the flank of a seamount at the crest of the East Pacific Rise[1], and from the Galapagos Spreading Center[5]. These deposits have been attributed to precipitation from hydrothermal fluids which gained high metal contents during interaction of seawater with basaltic rock at elevated temperatures. Although not conclusive, this type of genesis is more consistent with the geochemical and geological evidence than is a diagenetic formation by metal remobilisation and subsequent precipitation within sediment or formation by direct precipitation from seawater. Experimental evidence[7,8] showing that seawater becomes greatly enriched in Fe and Mn during interaction with fresh basalt at elevated temperatures seems to support the hydrothermal origin.

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Table 1 Chemical composition of crust from station 1052 compared with other reported hydrothermal deposits and an average Pacific manganese nodule

Sample	Location	Fe	Mn	Ni	Co	Cu	Zn	Fe/Mn
		(% by weight)			(p.p.m.)			
1052 (0–3 mm)	Hess Deep	21.8	16.7	1,950	508	735	543	1.30
D6 (0–3 mm) (data from ref. 6)	Galapagos Spreading Center	0.016	52.7	100	< 23	43	105	0.0003
T3-72D 253-13-21 (0–5 mm) (from ref. 2)	Mid-Atlantic Ridge (~26 °N)	0.011	39.2	100	18	12	—	0.0003
AMPH D2 (from ref. 3)	Flank of East Pacific Rise	32.5	1.94	400	35	74	—	16.7
Average nodule	Pacific	14.3	19.7	7,220	3,810	3,660	—	0.72

These ferromanganese deposits are characterised by low Th contents, high apparent rates of accumulation, and immediate proximity to sources of submarine volcanism. Samples from the TAG area[2,5] and the Galapagos Spreading Center[6] are further characterised by extremely low Fe and high Mn contents. Conversely, other suggested hydrothermal deposits are extremely Fe-rich and very low in Mn. The AMPH D2 deposit from a seamount on the flank of the East Pacific Rise, for example, has a Fe/Mn ratio of 16.7 (ref. 3). Unconsolidated metalliferous sediment such as that found along the crest of the East Pacific Rise generally have Fe/Mn ratios close to 3.0. Bonatti[9] suggests that the wide range of Fe/Mn ratios in these hydrothermal deposits is probably caused by drastic fractionation of the two elements, both before and after discharge of the metal-bearing solutions on to the sea floor. We report here on the geochemistry of a ferromanganese crust which is compositionally intermediate between the Mn-rich[2,5,6] and Fe-rich hydrothermal deposits[3,9]. We present evidence to demonstrate that this deposit must have formed at a substantially higher rate than deep-sea manganese nodules which are similar in their Fe/Mn ratios.

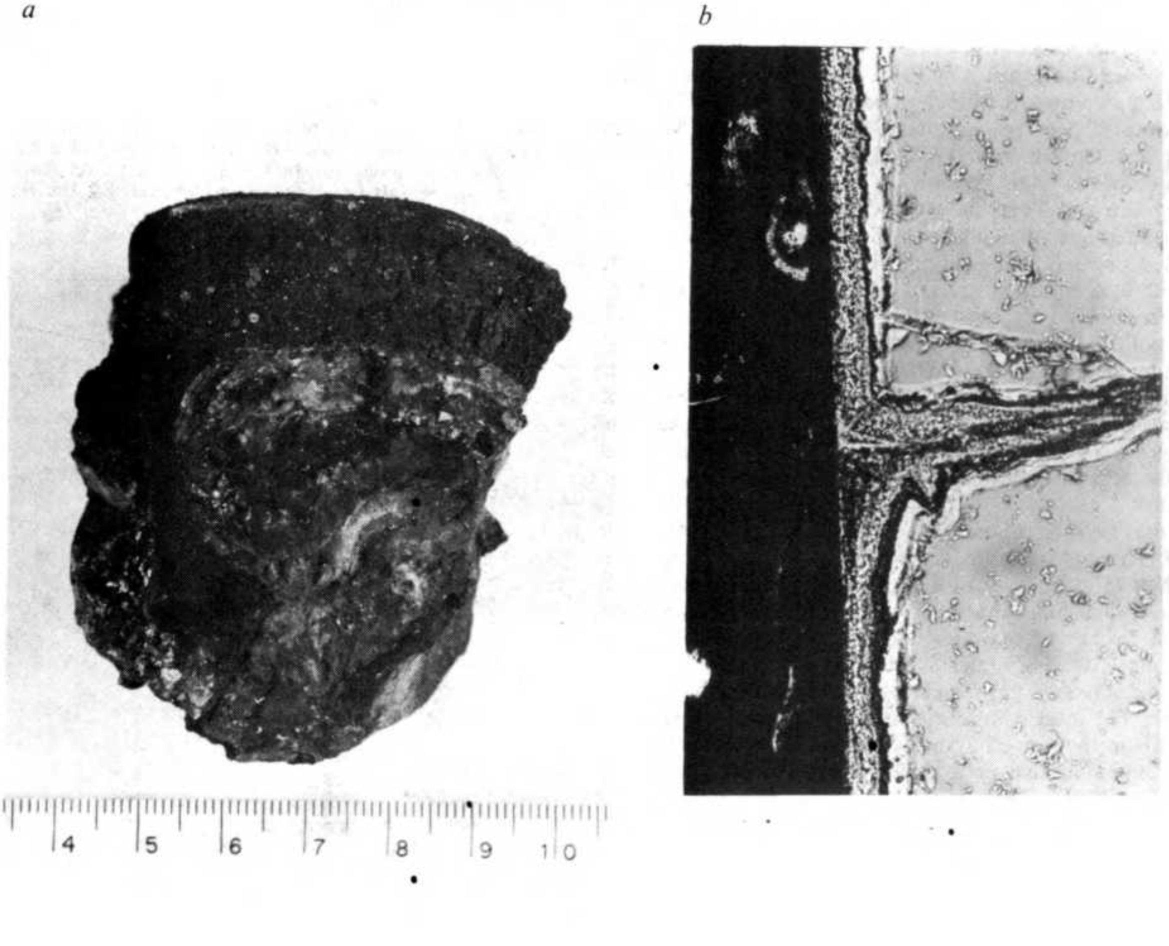
Our sample was collected at station 1052, on cruise 14 of the USSR RV Dmitry Mendeleev, at a depth of 3150 m, at 2° 17.8′N and 101° 1.5′W. The station is located in the axis of the Galapagos Spreading Center, just east of its junction with the East Pacific Rise. This area is known as Hess Deep[10] because of its relatively great depth for an oceanic rise system.

Chemical composition

The sample which we analysed consisted of 16 mm of ferromanganese material encrusting fresh basaltic glass (Fig. 1). Table 1 shows the chemical composition of the crust from station 1052 as compared to other reported hydrothermal deposits and to an 'average' Pacific manganese nodule. Analyses were performed by instrumental neutron activation analysis except for Cu and Ni which were determined by atomic absorption. Microprobe analyses of the underlying glass reveal typical ocean theolitic composition with low alkalis and a SiO_2 content of about 50%.

This sample is unlike the Mn-rich deposits from the TAG area and the Galapagos Spreading Center, having a much higher Fe and trace metal content. It is not as Fe-rich, however, as the iron deposit AMPH D2 from the flank of the East Pacific

Fig. 1 Photograph and microphotograph of thin section cut from sample 1052. Scale in (*a*) is in cm; the width of (*b*) is equivalent to approximately 1 mm.



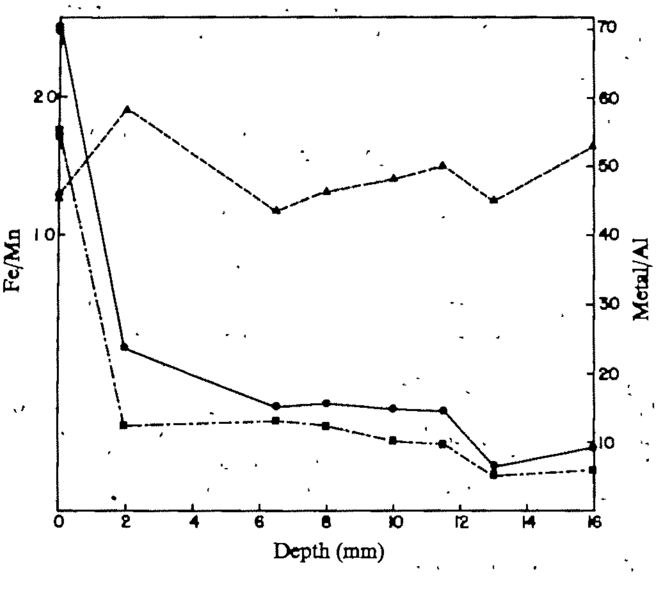


Fig. 2 Fe/Mn (▲), Fe/Al (●) and Mn/Al (■) ratios as a function of depth in ferromanganese crust as determined by microprobe analyses.

Rise. The minor metals reported in Table 1 for our Fe–Mn crust are all within the reported ranges for these metals in manganese nodules. The concentrations of Ni, Co and Cu, however, are near the minimum values found in nodules and cannot be described as typical. The only mineral detected in the crustal portion of our sample by X-ray diffraction procedures was δ-MnO_2.

Scott *et al.*[3] suggested that the extreme fractionation of Mn from Fe in deposits from the TAG area may result from the different solubilities of oxides of iron compared with Mn during oxidation by dissolved oxygen[11]. The reasoning is that Mn tends to be transported further from the source whereas Fe is precipitated at lower levels as oxides or sulphides. If this type of situation is representative of metal transfer in hydrothermal areas of the sea floor, then we may have, by chance, sampled an intermediate zone where both Fe and Mn are being deposited simultaneously. Scott *et al.*[5] have shown that the Mn-rich hydrothermal deposits from the TAG area are later covered by a more Fe-rich material, possibly of hydrogenous (seawater) origin. Moore and Vogt[4] have shown the same relation for a sample located near the Galapagos Spreading Center.

Figure 2 presents compositional data obtained at different levels within a polished section of 1052 using an electron microprobe. The Fe/Mn ratio remains between 1 and 2 throughout, but both the Fe/Al and Mn/Al ratios have a steep gradient in the outer portion of the crust. These metal/Al ratios reach levels on the exterior similar to those reported for EPR crest metalliferous sediments[12]. The reason for these steep gradients is not clear, although the concentration of volcanic debris (especially laths of plagioclase) was higher toward the interior of the crust.

Like the Fe/Mn ratios, the concentration of rare earth elements (REE) is uniform. The Ce/Fe and Eu/Fe ratios vary by less than 25% for seven of the layers analysed. The curve for the average REE concentrations, normalised to shale, is similar in pattern to that of seawater and crestal sediment from the East Pacific Rise (Fig. 3). This pattern is characterised by a negative Ce anomaly and enrichment of heavy REE relative to light REE. The total concentration of REE, however, is quite high and is in the range of concentrations for ferromanganese nodules, representative of a slowly accumulating manganese deposit. The shape of the curve, however, differs markedly from that of nodules. The curve for Pacific Ocean nodules which give similar X-ray diffraction patterns, usually have a positive Ce anomaly and slight depletion of the heavy (REE) Yb and Lu.

Growth rates

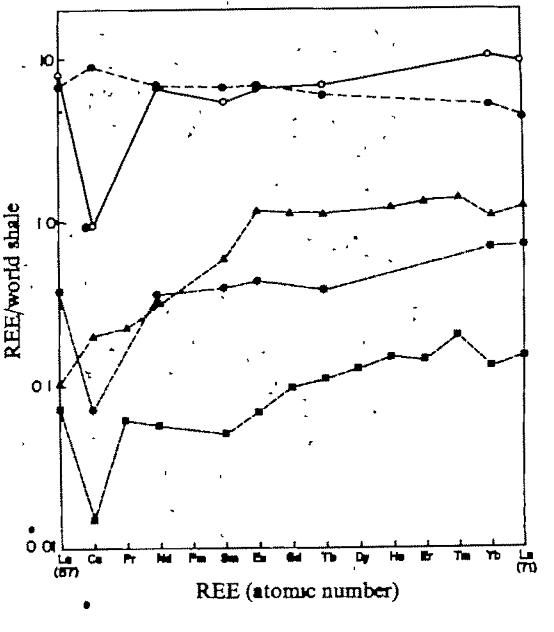
To assess the rate of growth of this deposit, uranium-series isotopes were measured in six contiguous layers throughout the crust. Separation, purification and counting of U and Th isotopes involved standard techniques[13]. The individual sections of the crust were removed by scraping the material from a measured area, weighing the material recovered, and calculating the depth based on an assumed density of 1.96 g cm^{-3} (ref. 14). Because of the configuration of this particular sample (Fig. 1), it was possible to measure directly the thickness of each interval by measuring the remaining thickness after each section was scraped away. The agreement between those two estimates was quite good, and it is estimated that the accuracy of the depth intervals is better than ±10%.

When the uranium-unsupported ^{230}Th activity is plotted relative to depth within the crust, a steep exponential curve results (Fig. 4*a*). If a constant rate of deposition of the ferromanganese material and a constant flux of ^{230}Th are assumed, and if the decline of the excess ^{230}Th is due solely to radioactive decay, we can estimate the rate of accretion of this material by dividing the depth interval over which half the activity of ^{230}Th disappears by the half life of ^{230}Th (75,200 yr). The resulting rate, 47.9 mm per 10^6 yr, is about one order of magnitude higher than typical rates deduced for deep-sea manganese nodules[15]. Although this rate is relatively high, there are several lines of evidence which indicate that, in fact, it must have grown considerably faster.

The assumption that the ^{230}Th gradient is due solely to radioactive decay is suspect because of the strong ^{232}Th gradient measured in the outer 8 mm of the crust (Fig. 4*a*). The slope of the ^{232}Th activity in this portion of the crust is so similar to that of ^{230}Th that when the excess ^{230}Th is normalised to ^{232}Th, there is virtually no slope (Fig. 4*b*). The deposit would have had to accrete at a minimum rate of about 100 mm per 10^6 yr to obscure a gradient in the $^{230}Th/^{232}Th$ ratio.

Another inconsistency in the uranium-series data concerns the isotopic composition of uranium throughout the thickness of the crust. The $^{234}U/^{238}U$ activity ratio for five of the six intervals analysed (Fig. 4*b*) is within analytical error of that in modern-day ocean water[16]. If this crust had accreted at an

Fig. 3 Rare earth elements (REE) in crustal portion of 1052 (○) and other marine phases normalised to shale. The curve for 'ferromanganese nodule' (●) is an analysis[13] of a nodule with similar mineralogy (δ-MnO_2) as the crust from station 1052. The ridge basalt (▲) analysis is from ref. 24, the East Pacific Rise (★) sediment from ref. 25, and seawater (■; data × 10^6) from ref. 26.



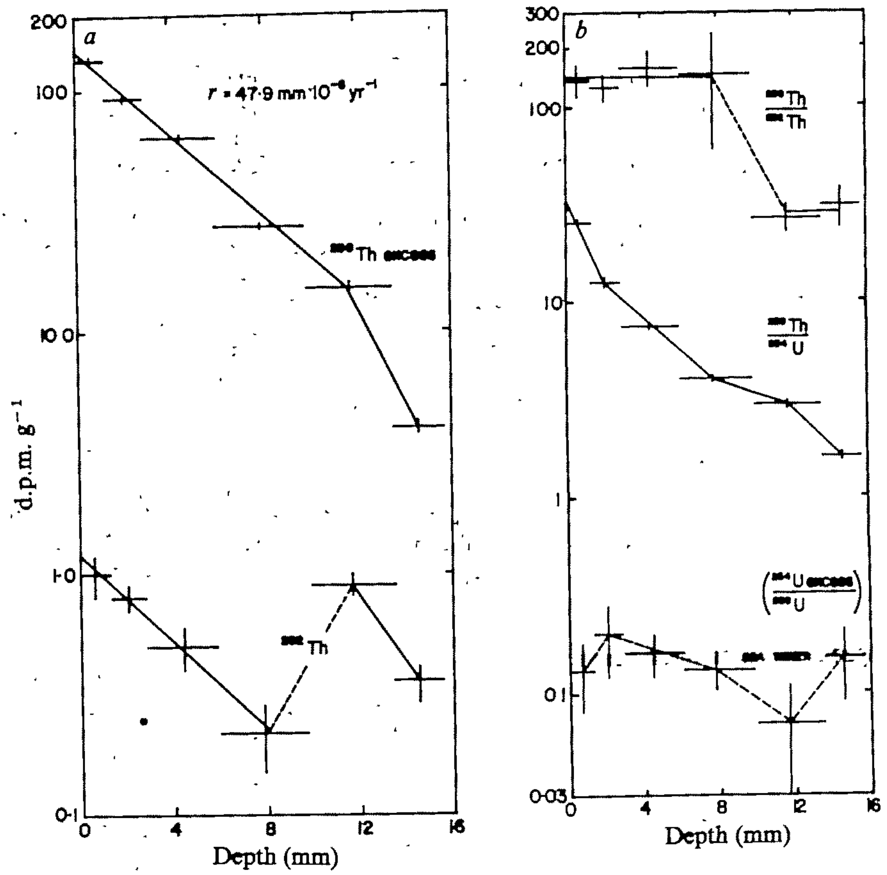


Fig. 4 *a*, Uranium-unsupported ^{230}Th activity and activity of ^{232}Th plotted against depth in ferromanganese layers of 1052. *b*, Activity ratios of ^{230}Th/^{232}Th, ^{230}Th/^{234}U and ^{234}U excess/^{238}U as a function of depth of ferromanganese in sample 1052. Horizontal error bars indicate range in thickness of layer sampled. Vertical error bars indicate 1σ errors as calculated from counting statistics.

average rate of 47.9 mm per 10^6 yr, the age of the base of the crust would be approximately 320,000 yr. If the uranium in the deposit had been derived from seawater and remained a closed system throughout its history, we would expect a ^{234}U/^{238}U activity ratio of about 1.06 for the basal layer of the crust. This is clearly not the case, and requires that we either had open exchange of uranium between the ferromanganese and seawater or the deposit is quite youthful.

Although it is difficult to reconcile the radiochemical data in terms of a slow rate of growth, we cannot conclusively demonstrate that the deposit formed at a faster or slower rate than calculated here, without an independent assessment of the age of the deposit. Because a well-developed zone of palagonite was present between the fresh basaltic glass and the ferromanganese, we tried to date the glass by hydration-rind techniques. This method, known to archaeologists as 'obsidian dating'[17], has also been applied to geological problems[18]. Because of the relative compositional uniformity of submarine basaltic glasses and the very constant ambient temperatures of oceanic bottom waters, the dating of submarine glasses by this technique seems to be reliable[19,20]. The principal assumption of the hydration-rind method is that the rate of submarine palagonisation of basaltic glass is constant, making it possible to obtain an age of the glass by simply measuring the extent of the hydration. An average rate of submarine palagonisation of 2.91 μm per 10^3 yr was obtained[20] by measuring the thickness of the hydration rind in submarine samples of known age. This rate has recently been confirmed on samples from the Mid-Atlantic Ridge, in which age was assessed by fission-track dating[21].

Using a thin section cut normal to the plane of growth of the ferromanganese material, several dozen traverses were made across the hydration rind using a microscope with a calibrated micrometer. This rind can be seen in the photomicrograph (Fig. 1*b*). The average thickness of the palagonite was 56±4 μm. This corresponds to a hydration age of 19,000±1,500 yr, much lower than the calculated minimum age of 320,000 yr, for the volcanic glass found by extrapolating the excess ^{230}Th data to the base of the crust.

Another way of estimating the age of this deposit from radiochemical data is to calculate an excess ^{230}Th 'exposure age'[14]. Assuming that the deposit contained no initial excess ^{230}Th but acquired its present-day content by exposure to normal ocean water, we can calculate the duration of this exposure by simply dividing the integrated content of excess ^{230}Th by its flux to the sea floor. The ferromanganese crust contains 145 d.p.m. ^{230}Th$_{ex}$ cm^{-2} and the flux of ^{230}Th is calculated to be 2.35 d.p.m. cm^{-2} per 10^3 yr per km of seawater on the basis of the normal uranium content of seawater with a 15% excess of ^{234}U and assuming quantitative removal of ^{230}Th produced from uranium. The 'exposure age' resulting from this calculating is 18,500 yr, in excellent agreement with the 'hydration age' of 19,000 yr.

Although our exposure age calculation produces an 'age' in apparent agreement with our hydration date, the cause of the exponential gradients of the Th isotopes must still be explained. It seems unlikely that ^{230}Th could diffuse over 15 mm in only 19,000 yr. Scott *et al.*[8] discussed the apparent inverse relationship between rates of manganese crust accumulation and content of trace metals. If the amount of thorium incorporated into a ferromanganese crust is also inversely proportional to its growth rate, then the gradients that we have measured could simply be a result of the accretion rate slowing down.

Our age estimates for this deposit (1052) are summarised below.

(1) Extrapolation of rate derived from excess ^{230}Th gradient:

Apparent rate of growth	= 47.9 mm per 10^6 yr
Thickness of crust	= 15.5 mm
Minimum age of glass	= 320,000 yr

(2) Hydration-rind dating of basaltic glass:

Rate of submarine palagonitisation	= 2.91 μm per 10^3 yr
Average thickness of palagonite rind	= 56 μm
'Hydration' age of glass	= 19,000±1,500 yr

(3) Time required to acquire integrated amount of excess ^{230}Th

$$\sum {}^{230}\mathrm{Th}_{ex} = \int_{0}^{\infty} {}^{230}\mathrm{Th}_{0}\rho \exp(-\lambda^{D})\mathrm{d}D$$

where $^{230}\mathrm{Th}_0$ = extrapolated surface activity of excess ^{230}Th (d.p.m. g^{-1}); ρ = bulk density (g cm^{-3}); D = depth in crust; $\lambda = 0.693/D^{\frac{1}{2}}$ ($D^{\frac{1}{2}}$ = depth at which activity of excess ^{230}Th equals $\frac{1}{2}$ that of surface value) and ^{230}Th flux = 2.35 d.p.m. cm^{-2} per 10^3 yr per km seawater. Depth of crust = 3,340 m.

For 1,052: $\Sigma^{230}\mathrm{Th}_{ex}$ = 145 d.p.m. cm^{-2}. Therefore, 'exposure' age = 18,500 yr.

Based on the correspondence between the ages derived from hydration-rind and ^{230}Th flux techniques, and the fact that the ^{230}Th distribution in the ferromanganese crust precludes radioactive decay being the only process affecting the ^{230}Th gradient, the lower age estimate for this sample would seem to be the more accurate estimate. If the age of the volcanic glass is actually about 19,000 yr, the minimum rate of accretion of the Fe–Mn oxides would be about 800 mm per 10^6 yr. In fact, it must have been even faster, since we have calculated that more than 18,000 yr of exposure to seawater would be necessary for accumulation of the excess ^{230}Th that is present in the crust today. Volcanism could have supplied some of the ionium, but this is unlikely since other hydrothermal deposits sampled from oceanic ridges have been shown to contain no excess ^{230}Th (refs 2 and 6).

Conclusions

Our results show that in our sample from the Hess Deep, first, a ferromanganese crust has been deposited without severe fractionation between Fe and Mn. Second, the trace metals Ni, Co and Cu are low compared with typical manganese nodules but the content of REE is about the same. Third, the REE pattern, normalised to shale, is very similar to the seawater pattern. Fourth, the major assumptions in applying U-series dating techniques for assessing rates of growth seem to fail in this case and we conclude that the deposit must have accreted considerably faster than that indicated by the excess ^{230}Th method.

On the basis of these results, we suggest that Fe and Mn as well as some of the trace metals in this crust were deposited from hydrothermal fluids while REE were scavenged from seawater.

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Electrophoresis along cell membranes

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Bioelectric fields may segregate charged components floating in the plasma membranes of cells by a process of electrophoresis along the membrane. Molecules in cell membranes may be sorted to different portions of the cell surface by such electrical gradients. We present here a theory to support this hypothesis.

MANY molecules and particles are freer to move along the outer membrane of cells than out of this membrane[1,2]. Many of the externally protruding components bear a high negative charge density[3–6]; whereas lecithin (and sphingomyelin) have a substantial positive charge at the calcium ion concentrations typical of extracellular fluids[7,8], and neutral lipids such as cholesterol and certain glycolipids are also common in the outer leaflets of cell membranes[9,10]. If lateral mobility and charge heterogeneity among these membrane components are sufficient, application of a steady field to a cell should substantially redistribute them within the plane of the plasma membrane (Fig. 1). This process may be called surface, lateral, or perhaps perimembrane electrophoresis. I present here a study of the basis and implications of this idea.

Equilibrium electrophoresis along the membrane of a spherical cell

A complete theory of lateral electrophoresis is analogous to the theory of sedimentation. Both the equilibrium with back diffusion and the approach to this equilibrium must be considered. For the present purpose, only the simpler and apparently more useful equilibrium theory will be presented.

Consider a spherical cell which bears a population of one species of negatively charged molecules or particles on its surface, and which is placed in a uniform field (Fig. 1). Neglect any interactions (other than hydrodynamic drag) between various surface components. Allow them to come to equilibrium between electrophoresis which tends to concentrate them at the positive pole and back diffusion. It will be shown that the equilibrium surface concentration, C, is given by

$$C = \overline{C}[\varepsilon \cdot \mathrm{csch}(\varepsilon)]e^{\varepsilon \cos \theta}, \qquad (1)$$

where $\overline{C}$ = the particles' average concentration, $\varepsilon = (m/D) \cdot (\overline{V}/2)$, m = electrophoretic mobility, D = diffusion constant,

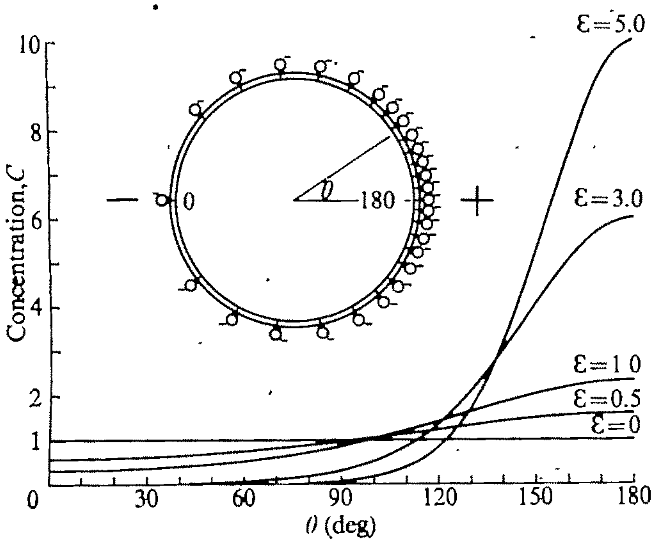


Fig. 1 Distributions of mobile charged particles on the surface of a sphere at electrophoretic equilibrium. Curves plot equation (1).

and $\bar{V}$ = voltage difference established across the sphere by the field. This is plotted in Fig. 1.

The particles are at a true equilibrium between several forces produced by the external field and Brownian movement, so it might be supposed that a Boltzmann distribution could be applied. Their counter ions are not, however, in equilibrium but in a steady state, so this most direct method cannot be used. Instead one writes the equation for a balance between electrophoretic and diffusional transport

$$mC(\mathrm{d}V/\mathrm{d}S) = D(\mathrm{d}C/\mathrm{d}S) \tag{2}$$

A sphere is known to distort an otherwise uniform field so that the potential just outside its surface, V, remains proportional to cosθ (ref. 11). Hence

$$V = (\bar{V}/2)\cos\theta \tag{3}$$

Substituting equation (3) in (2) and integrating yields equation (1).

Exactly the same derivation holds for the distribution of particles attached to the inner surface of a cell's membrane and concentrated by a uniform internal field, except that in this internal case $\bar{V}$ would refer to the transcytoplasmic potential difference. A uniform internal field might be set up by either a uniform external one[11] or by a cosine varying current source in the cell's own plasma membrane[12].

For comparison with cells' galvanotropic responses (or perhaps other polar responses) it is helpful to characterise the degree of polarisation of each of these equilibrium distributions[1] by some parameter, φ. The tropistic responses of cell populations are commonly characterised by the weighted cosine of the distribution of outgrowth angles (13)

$$V_1 = \Sigma n \cos\theta / \Sigma n \tag{4}$$

where n is the number of outgrowths at an angle, θ.

Hence we define φ in an analogous way

$$\varphi = \int C \cos\theta \, \mathrm{d}s / \int C \, \mathrm{d}S \tag{5}$$

Note that if the outgrowth angles or surface particles were all concentrated at one pole or the other, V_1 and φ would be +1 or −1 whereas if they were evenly distributed, these parameters would have a value of zero.

Integration of equation (5) yields

$$\varphi = \mathit{ctnh}\,(\varepsilon) - \varepsilon^{-1} \tag{6}$$

Use of Pierce's series[14] number 793 yields

$$\varphi = \varepsilon/3 - 0.133\,\varepsilon^3 + 0.127\,\varepsilon^5 \ldots . \tag{6a}$$

This suggests that for $\varepsilon < 1$, φ is usefully approximated by

$$\varphi \simeq \varepsilon/3 = (m/D)(\bar{V}/6). \tag{6b}$$

Equations (6) and (6b) are plotted in Fig. 2.

Expected polarisability of real membrane particle distributions

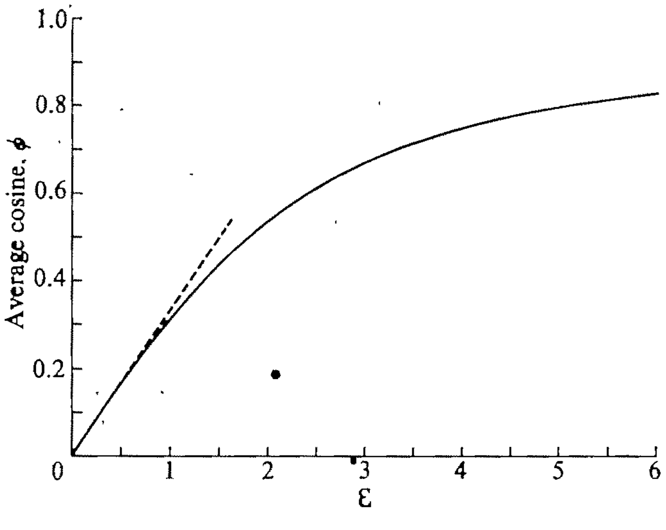
Let us characterise the polarisability of a particle distribution on a cell by the voltage $\bar{V}_{0.1}$ required to induce a polarisation of 0.1. From equation (6b) one gets

$$V_{0.1} = 0.6\,(D/m) \tag{6c}$$

To estimate this voltage, one must estimate the diffusion to mobility ratio, D/m of the particles being redistributed. We will show below that the larger and more highly charged particles found in cell membranes are likely to have D/m values of about a millivolt. So we predict that long-lasting fields, whether endogenous or imposed, are likely to bring about significant electrophoresis along cell membranes when the voltage drop per cell is only one to a few millivolts.

To estimate D/m values, we will first show that D/m is independent of the drag, f, which they encounter. This drag is an estimated 100–1,000 times greater than such particles would meet if they were only hindered by the extracellular medium[8]. If this greater drag were exerted by the viscosity of the medium which fills and surrounds their electrical double layer, then D and m are both well known to vary with f^{-1}. Hence D/m would be independent of f. It is likely, however, that this extra drag is exerted on a sort of molecular clog, that is, a part or extension of each particle that lies within the lipid bilayer and is not surrounded by a field-sensitive double layer; whereas the electrical pull is exerted on each particle's 'sail', that is, its part that extends outside of the bilayer in the external aqueous phase (Fig. 1). Then m would equal F, the net electrophoretic force per field (which acts on the sail), divided by the drag which acts on the clog. Henry's analysis[15], directly confirmed by Sumner and Henry's experiment[16], shows F to be unchanged whether the particle is free or tethered. Hence m is inversely proportional to the drag wherever it originates. Since this also holds for D (ref. 17), D/m is again independent of the drag.

Fig. 2 Average cosines of the distributions shown in Fig. 1. The curve plots equation (6); the dashed line, equation (6b).



Since D/m values are independent of the drag, we can use the values known for molecules or particles immersed in the usual 1-cP aqueous media. In media with this viscosity (and an ionic strength of 0.05 M) most biological molecules or particles have electrophoretic mobilities in the range from 1 to 3, or occasionally up to 5×10^{-4} cm s^{-1} (V cm^{-1})$^{-1}$ (refs 5, 18). Electrophoretic mobilities are independent of the component's radius, but diffusion constants are inversely proportional to it. So to estimate how small a voltage drop per cell may be expected to bring about significant redistribution, let us consider relatively large membrane particles. Let us consider spherical particles with 10-nm diameters since the larger particles seen in freeze-fracture electron micrographs of plasma membranes are of about that size and shape[19–22]. In 1-cP media, such 10-nm spheres have a diffusion constant of 4×10^{-7} cm^2 s^{-1} (ref. 24). Then considering particles with a mobility value of 3×10^{-4} units, which is towards the upper end of those usually found, one obtains a D/m value of 1.3 mV and applying equation (6) we infer that the distribution of such particles would be one tenth to one half polarised by a steady voltage drop of only 0.8–4.0 mV.

Application of this equilibrium theory is restricted to the action of sufficiently long-lasting fields. The time needed is of the order of L^2/D where L is the cell's pertinent diameter and D the segregated components diffusion constant. The larger mobile components of cell membranes tend to have diffusion constants in the vicinity of 10^{-9} cm^2 s^{-1} (ref. 2), so for a 30-μm (that is, 3×10^{-3}-cm) cell, the equilibrium approach time might be about 10^4 s, or 3 h. When one considers that the bioelectric fields associated with growth and development generally last for hours to days or even weeks (that is, 10^4 to 10^6 s), one concludes that an equilibrium theory may be widely relevant.

The accompanying paper, by Poo and Robinson[25] seems to directly demonstrate the predicted phenomenon of lateral electrophoresis along the surface of explanted, embryonic muscle cells exposed to an artificially imposed field. Moreover, a number of galvanotropic responses of isolated plant cells to artificial fields occur in response to such small voltage drops as to suggest mediation by lateral electrophoresis[26–28].

Natural fields along cell membranes

Perhaps the most obvious place where large endogenous voltage drops occur along cell membranes is along the outside of the lateral membranes of cells in various metazoan epithelia. Various mature epithelia are well known to maintain voltage drops of 30–100 mV or more across themselves[29]. Furthermore, there are several reports of 3–10 mV potential drops across embryonic cell layers, for example, the mammalian blastocyst wall[30], a fish gastrula wall[31], and the chick chorioallantoic membrane[32]. A second place where good evidence for significant steady potentials drops along cell membranes can be cited is along the outer plasma membranes of epidermal cells in the coleoptiles of oat seedlings. A voltage drop of at least 40 mV (refs 33, 34) is measured along the axis of these organs and hence across about 30 cells in series[35]; whereas up to 80 or 90 mV appears across the axis in response to a gravitational stimulus[36,37] and thus across about 100 surface cells in series[35]. Thus, in both directions, about 1 mV per cell is present.

There are, moreover, at least three instances in which there is evidence that cells generate large enough cytoplasmic fields within themselves electrophoretically to segregate components of the inner leaflet of the plasma membrane. Woodruff and Telfer have quite directly measured a voltage drop of about 10 mV across the cytoplasmic bridge joining a developing insect oocyte and its nurse cell[38]; Hagins has measured a steady dark current outside of rat visual rod cells and infers an internal current sufficient to maintain a steady difference of about 1.6 mV along the interior of the cell[39]; while Robinson and Jaffe have measured a calcium current through developing fucoid eggs which might maintain the order of a millivolt difference across the interior of the cell[40,41].

I conclude that endogenous voltage gradients sufficient to bring about electrophoresis along cell membranes are very widespread in living organisms.

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Electrophoresis of concanavalin A receptors along embryonic muscle cell membrane

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Fluorescent concanavalin A (con A)-labelling showed that an electric field of 4 V cm^{-1} grossly redistributed con A receptors along the plasma membranes of living muscle cells within 4 h. This field produced a voltage drop of 12 mV across these 30 μm-wide cells. The movement of receptors was independent of cell metabolism and seemed to be electrophoretic in nature.

STUDIES of the cell membrane have demonstrated the dynamic nature of membrane constituents[1–5]. Both proteins and lipids may undergo rotational and translational diffusion in the plane of the membrane[6]. In the accompanying paper[7], Jaffe demonstrates on theoretical grounds that small voltage

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differences (of the order of 1 mV across a cell) applied parallel to the surface of cells should be effective in redistributing charged, mobile entities in the plane of the membrane. We have placed cultured embryonic amphibian muscle cells in a field of 4 V cm^{-1} and examined the subsequent distribution of the receptors of concanavalin A (con A). We found, using fluorescein isothiocynate (FITC) labelled con A, that these receptors become asymmetrically arranged within a few hours. The receptors (presumably glycoproteins) accumulated on the side of the cells nearer the negative pole in a way consistent with a passive electrophoretic mechanism.

Muscle cells were obtained from the neural tube region of 1-d-old *Xenopus laevis* embryos (stage 18–19 by the criterion of Nieuwkoop and Faber[8]). After removal of the vitelline membrane, the neural tube and the underlying mesoderm were cut from the embryo in Ca^{2+}-, Mg^{2+}-free Steinberg's solution containing 0.4 mM EDTA (ref. 9). When the cells became dissociated, they were transferred to a shallow, rectangular glass chamber which was precoated with poly-L-lysine. The cells were cultured in Steinberg's solution supplemented with 10% Leibovits medium (L15, GIBCO) and 5% foetal calf serum (GIBCO). The *p*H of the culture medium was 7.8. Each culture contained various cell types from a single embryo and was incubated at room temperature (about 25 °C). Although neurones and other cell types survive for a while, after 2 d the predominate cells in the culture were single, spindle-shaped, striated muscle cells. These cells responded to 0.01% carbachol (Sigma) by contracting from both ends into a sphere.

The 0.5-mm deep chamber was covered with a coverslip before application of the electric field, thus providing a defined geometry. The current was applied through a pair of 8-cm-long bridges filled with Steinberg's solution gelled with 2% agar. These agar bridges isolated the cultures from contamination by the Ag–AgCl electrodes, to which a regulated power supply was connected. The applied fields were calculated from the current (1.5 mA usually), the medium's conductivity (130 Ω cm), and the chamber's cross section (0.05 cm^2). The usual current of 1.5 mA produced a field of 4 V cm^{-1} in the chamber.

After the field was shut off, the cells were cooled to 0–5 °C, the culture medium was washed off with Steinberg's solution, and then Steinberg's solution containing 200 μg ml^{-1} FITC–con A (Miles) was introduced into the chamber. After 10 min, the FITC–con A was removed with Steinberg's solution. In some cases the cells were then fixed in cold acetone (−5 to 0 °C) and preserved in 90% glycerol; in other cases, the living cells were examined by conventional and fluorescent microscopy. We found that glutaraldehyde fixation caused marked autofluorescence in these muscle cells, and glutaraldehyde thus was an unsuitable fixative.

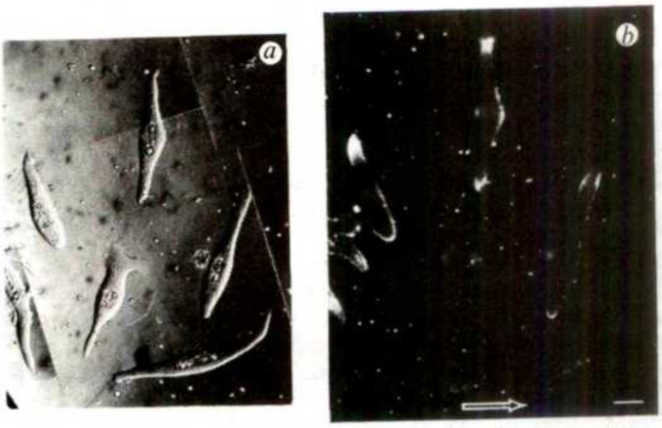

Fig. 1 FITC– con A-labelled living muscle cells observed by *a*, light miscroscope fitted with Normarski optics and *b*, dark-field fluorescence miscroscope. Embryonic muscle cells in monolayer culture exposed to a uniform electric field (4 V cm^{-1}) for 4.5 h before staining. Fluorescent 'ring' showed asymmetry for nearly all cells in the culture, with more stain accumulated towards the negative pole of the field (to the right of the figure). The brighter stain near the tips of some cells was due to partial contraction of the cells which we assume caused folding of the membrane resulting in higher concentration of con A receptors. Fluorescent spots in the background and on the surface of some cells were due to stained cell debris. Direct visual comparison of the fluorescence intensity on two sides of the cells were carried out for all cells which were aligned relatively perpendicular to the direction of the field. Labelling was done at 0–5 °C for 10 min. Bar: 30 μm.

Table 1 Con A receptor distribution in electrical field

Duration of electric field application* (and voltage drop across each cell)	No. of cells examined†	No. of cells showing asymmetry in the fluorescence 'ring' staining					Description of the asymmetry in fluorescence
		Towards (−) pole		Towards (+) pole		Indeterminant	
0	53	7	(13%)	10	(19%)	36	no detectable asymmetry
30 min (12 mV)	77	18	(23%)	16	(21%)	43	no detectable asymmetry
1.5 h (12 mV)	103	67	(65%)	11	(11%)	25	weak but definite asymmetry
4.5 h (12 mV)	86	70	(81%)	3	(3%)	13	striking asymmetry
14 h (12 mV)	27	25	(92%)	0	(0%)	2	striking asymmetry
24 h (0.6 mV)	60	27	(45%)	9	(15%)	24	weak but definite asymmetry

*All data for 12 mV voltage drop are from the same experiment; data for 0.6 mV voltage drop are from two other experiments.

†Cells were randomly sampled with only one criterion: their long axes were aligned within 30 degrees with respect to the direction normal to the field direction, so that the fluorescence staining on two sides of the cell could be compared (see text for detail).

The fluorescent microscopy was done using a standard Zeiss microscope fitted with a dark-field condenser. The excitation source was a Zeiss HBO 200W/4 lamp. Exciting light was passed through a heat-absorbing filter and a BG 12 blue filter, and emitted light was filtered with a No. 50 barrier filter.

Redistribution of con A receptors

In control cultures, where no electric field was applied, the FITC–con A-labelled cells showed uniform staining with a bright ring around the cell periphery since the surface is seen edge-on there (Fig. 2*a*). This ring was our most reliable and sensitive indicator of receptor redistribution. The labelling was specific, since it was completely blocked by 0.1 M α-methyl-D-mannoside, one of the sugars for which con A is specific[10]. Further, most of the label on prelabelled cells was removed by applying 0.1 M α-methyl-D-mannoside for 10 min.

When an electric field of 4 V cm^{-1} was applied to cell cultures for 4.5 h, nearly all cells whose long axis was perpendicular to the field showed a striking asymmetry in their ring staining pattern, with more label appearing on the side of the cells facing the negative electrode (Fig. 1*b*). Bright-field microscopic observations (both phase-contrast and Nomarski) were made on the same cells and no morphological difference corresponding to the observed asymmetry in fluorescence staining could be seen (Fig. 1*a*). Cells that were oriented with their long axes parallel to the field also showed some accumulation of label at the negative-facing tip, but this effect was much smaller and harder to score than the asymmetry along the short axis.

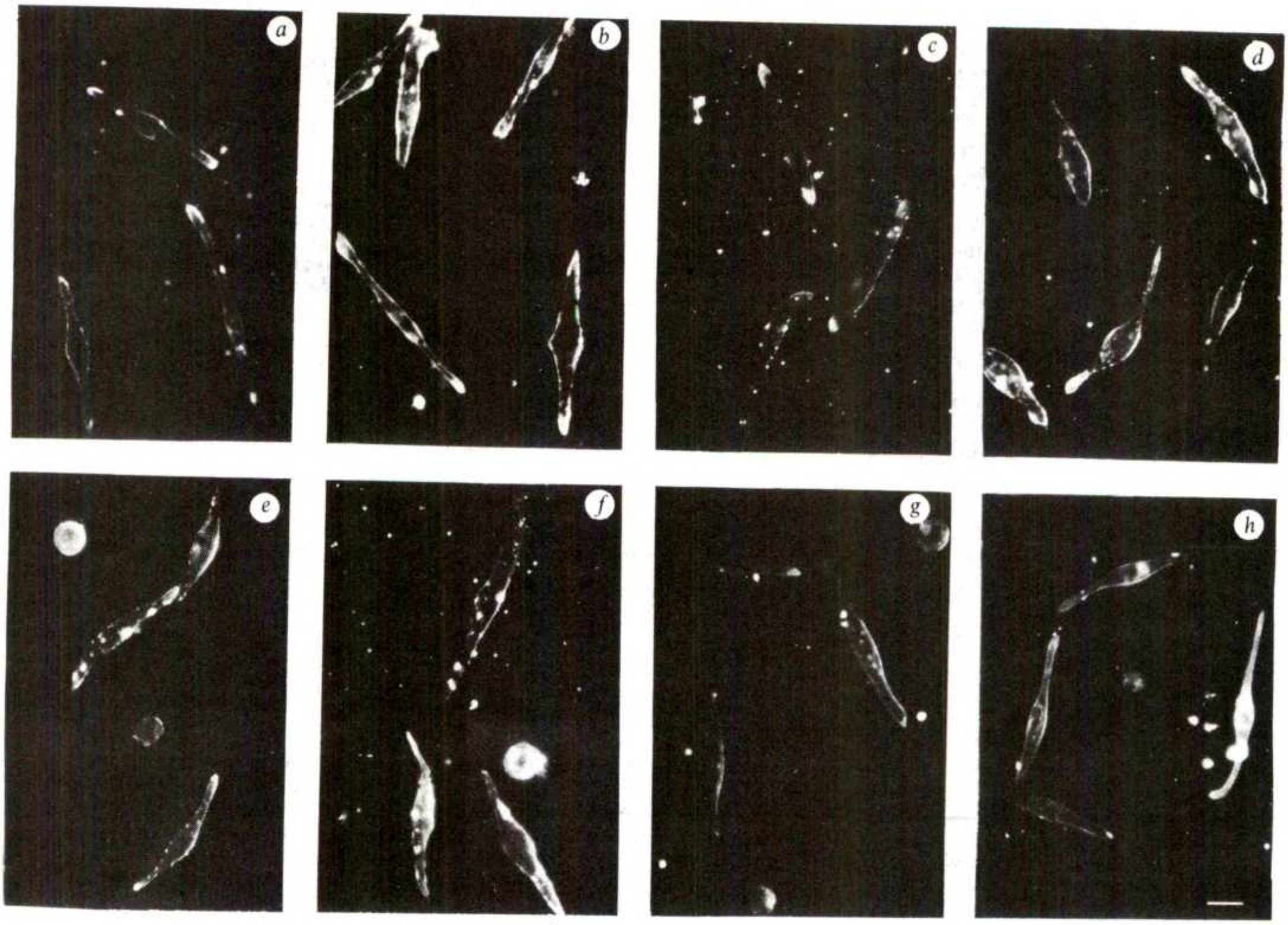

Fig. 2*a*, Control culture, where no field has been applied, FITC–con A-labelled cells showed uniform staining with a bright ring around the cell periphery. *b*, *c*, *d*, Cells were exposed to an electric field of 4 V cm^{-1} for 30 min, 1.5 h, and 4.5 h, respectively, before FITC–con A labelling. *e*, Cells were preincubated in Steinberg's solution containing 10^{-2} M sodium azide and 10^{-3} M dinitrophenol for 2 h. A field of 4 V cm^{-1} was then applied for 2 h before labelling. Both field application and FITC–con A labelling were carried out in the presence of these compounds. *f*, Cells treated as in (*e*) except the Steinberg's solution contained 10^{-2} M sodium arsenate, 10^{-3} M sodium fluoride, and 10^{-3} M dinitrophenol. *g*, Cells treated as those in (*d*) except Ca^{2+} was omitted from the medium during the application of the field. *h*, Cells treated as those in (*d*) except FITC-conjugated wheat germ agglutinin was used as a fluorescent label instead of FITC–con A. All cells were fixed with cold acetone (at 0 to +5 °C) after labelling. Negative pole of the field was on the right of the figure. Bar: 30 μm.

To investigate the time course of the redistribution, we applied the same field (4 V cm^{-1}) to a group of cultures for 30 min, 1.5 h, 4.5 h, and 14 h. The asymmetry in fluorescence staining on cells which had their long axes approximately perpendicular to the field was then scored (see Fig. 2*a*–*d*). We found that the effect was clearly perceivable after 1.5 h in the field and that it did not seem to increase beyond 4.5 h. Table 1 shows data from one experiment; similar results were obtained in four separate experiments. We concluded that the con A receptor moves halfway across the surface of the 30-μm-wide muscle cell in 1–2 h.

After a field as small as 0.2 V cm^{-1} (corresponding to a potential difference of 0.6 mV across a 30-μm cell) was applied to a culture for 24 h, a detectable asymmetry in the con A receptors was found (see Table 1). Fields smaller than 0.2 mV cm^{-1} did not cause detectable redistribution.

We have considered the possibility that the apparent positive charge of the con A receptors was due to the polycation, poly-L-lysine, that was used to coat the surface of the glass on which the cells were grown. The cells grew well on clean, untreated glass, so the experiment was repeated in the absence of poly-L-lysine, with a field of 4 V cm^{-1}. The same obvious accumulation of the con A receptors on the negative side of the cell was seen after 4 h.

In two separate experiments, FITC-wheat germ agglutinin (WGA) was added to cells which were in a 4 V cm^{-1} field for 4.5 h. Some cells seemed to show a slight accumulation towards the negative side (Fig. 2*h*); however, a count of cells such as was done for Table 1 showed that the asymmetry was less than was seen for the con A receptors after 1 h in the field. The WGA receptor is thus either much less charged or much less mobile than the con A receptor.

Mechanism of receptor redistribution

We considered the redistribution of the con A receptors in an electric field to be a passive, electrophoretic phenomenon. As an alternative, however, the field might somehow align and activate a motive system in the cortex of the cell which then actively redistributes surface components, including the con A receptors. Since any such system would presumably require metabolic energy, we performed experiments in the presence of metabolic inhibitors. First, we preincubated the cells in Steinberg's solution containing both 10^{-2} M sodium azide and 10^{-3} M dinitrophenol, a treatment known to prevent con A-induced capping in other cells[11,12]. As a second, more drastic test, we preincubated other cells in a medium including 10^{-2} M sodium arsenate, 10^{-3} M sodium fluoride, and 10^{-3} M dinitrophenol for 45 min. This latter treatment blocked their response to 0.01% carbachol, a carbachol concentration which caused instantaneous contraction in control cultures. This indicated that the cellular energy supply for the contractile machinery had been exhausted. Cells so pretreated were then placed in an electric field (still in the presence of the respective metabolic inhibitors) and the distribution of the con A receptors examined as before. Neither treatment had any effect: the con A receptors still accumulated on the negative side with the same time course and to the same extent as in control cells (see Fig. 2*e*, 2*f*). Similarly, the presence of 5×10^{-6} M colchicine throughout the experiment had no effect on the redistribution. Moreover, when Ca^{2+} was omitted from the medium ($[Ca^{2+}] < 10^{-5}$ M) during the application of the field, the redistribution proceeded as in the normal Ca^{2+} medium (Fig. 2*g*).

We therefore believe that the observed redistribution of the con A receptors is a passive response of these molecules to the electric field. As will be discussed later, the estimate of receptor mobility from these data is consistent with the idea of electrophoretic movement of receptor molecules through an environment with a drag similar to the measured drag on other cell membrane constituents[6]. Moreover, if the redistribution of the con A receptors is a passive electrophoretic movement, removal of the electric field after the receptor is asymmetrically distributed should allow a relaxation of the asymmetry due to back diffusion. Preliminary results indicated that if cells were allowed to remain in the culture medium following the application of a field but before the application of FITC-con A, there was a partial relaxation of the asymmetry. Some recovery to the original uniform distribution was apparent after 3 h. About 50% of the cells examined, however, still showed a weak asymmetry in the receptor distribution even after 54 h. Similar results were obtained in three separate experiments. The characteristic $1/e$ time for the partial relaxation of asymmetry is about 10 h (range 5–15 h). Relaxation experiments have not been performed in the conditions inhibiting metabolism, since the cells die if treated with drugs for the longer times required in these experiments.

Discussion

An externally applied electric field may exert its action on cultured muscle cells in several ways. It may act by producing a cytoplasmic potential gradient. It may act by changing the membrane potential on the two sides of the cell. It may also act tangentially along the surface of the cell. In our experiments, the potential difference produced by the field across a perpendicularly aligned muscle cell (average width 30 μm) was about 12 mV. Since only a very small fraction of the external voltage drop would lie across the cytoplasm, the directed movement of cytoplasmic components which could in turn redistribute surface receptors seems quite unlikely. The membrane potential on the two sides of the cell facing the electrodes will be shifted by about 6 mV. It is not clear, however, how this steady shift in membrane potential could have caused the observed redistribution of the receptor molecules in the plane of the membrane. Rather, we propose that the primary action of the applied field is to act tangentially along the membrane[7,13]. An external tangential field would be experienced by the charged, protruding heads of membrane-bound molecules, and these molecules would thus be driven (to the extent that they are mobile) towards the anodal or cathodal side of the cell. Quantitatively, this mechanism is a plausible explanation of the redistribution of con A receptors which we have observed (see ref. 7).

A crude estimate of receptor mobility can be made from the data on the time course of the redistribution. We take 2 h as the time needed for a receptor molecule to move approximately half way across the width of the cell, that is, the average electrophoretic mobility of the receptor is about 15 μm per 2 h per 4 ($V\ cm^{-1}$), or about $5\times10^{-4}\ \mu m\ s^{-1}\ (V\ cm^{-1})^{-1}$, a value 2×10^5 fold smaller than the electrophoretic mobility of a typical globular protein in an aqueous medium. The viscous drag experienced by the receptor molecules undergoing electrophoretic movement in the plane of the membrane is thus similar to that imposed by a medium having a viscosity of about 20 P (since the viscosity of water is 1 cP), which is close to but higher than other estimates of membrane viscosity[6]. Experiments on the relaxation of the asymmetry after the field is turned off suggest a diffusion coefficient for lateral diffusion of the mobile unlabelled receptor of $3\times10^{-11}\ cm^2\ s^{-1}$ (range $2–6\times10^{-11}\ cm^2\ s^{-1}$), since the diffusion coefficient, $D=r^2/2t$, where r is the radius of the cell ($\sim$15 μm), and t is the $1/e$ relaxation time[14]. This is a value comparable to those reported for mobile membrane proteins diffusing in the plane of the membrane[5,6,15–18].

The accumulation of con A receptors on the negative side of these muscle cells indicates that the majority of these molecules are either positively charged or else less negatively charged than other displaceable membrane components. This agrees with the finding of Skutelsky and Farquhar that the con A receptors of developing red blood cells have relatively little affinity for positively charged colloidal ion particles[19].

Finally, we expect that the *in situ* electrophoresis of cell surface components demonstrated in this report will prove to be a useful technique for measuring receptor mobility in the plane of the membrane and for exploring the molecular properties of cell-surface receptors within their natural membrane environment.

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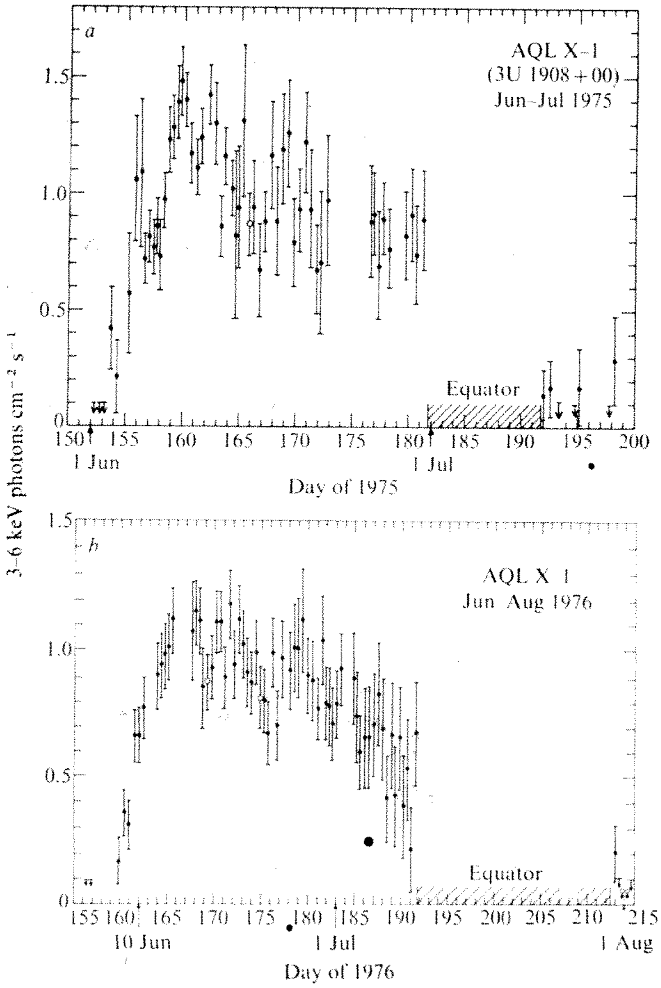
letters to nature

Recurrent X-ray outbursts from Aquila X-1

A HIGH degree of variability has been associated with Aquila X-1 (Aql X-1) (3U1908+00) since its occasional observation in early rocket surveys[1-3], and its appearance as a variable source of moderate brightness in the Uhuru catalogue[4] ($S \sim 0.07$–$0.2\, S_{\rm crab}$). Later observations of the source have been made from OSO-7 (ref. 5) during 1971–73 which suggest the existence of 'low' ($S \sim 0.02\, S_{\rm crab}$) and 'high' ($S \sim 0.5\, S_{\rm crab}$) intensity states, and from OAO Copernicus, where the source was reported at levels at least an order of magnitude below the Uhuru range during intermittent exposures over a 2-yr period through 1975 May (ref. 6). This apparent quiescent state was interrupted in 1975 June by a sudden flare[7] (factor of >20 increase) to the level of the Crab nebula. Observations of the declining source by the Sky Survey Instrument (SSI) on Ariel V revealed an ~10% sinusoidal modulation[8] (2–18 keV) at the period $P = 1.3 \pm 0.04$ d. By August the flux had decayed back to the pre-outburst intensity ($< 0.003\, S_{\rm crab}$) as measured by the SSI (ref. 9), which instrument observed the same low flux from Aql X-1 again in 1976 January. In 1976 June, the All-Sky Monitor (ASM) on Ariel V detected an additional outburst of this source[10], which was confirmed by the SSI (M. G. Watson, personal communication). We report here ASM observations of Aql X-1 obtained between the launch of Ariel V in 1974 October and 1976 November, and compare them with the previously cited additional data available. We suggest that the totality of these measurements imply that Aql X-1 may provide a plausible connection between dwarf novae and the very bright, long-duration transient X-ray source[11].

Fig. 1 ASM chronologies of the 1975 (*a*) and 1976 (*b*) flares of Aql X-1. The points are ~½-d averages, and the error bars are ±1σ (statistical error only). The shaded areas labelled 'equator' are times when Aql X-1 was close to the equatorial plane of the of the Ariel V satellite; during these times the source was inaccessible to the ASM, but observable by the SSI.



The proximity of Aql X-1 to the sources Ser X-1 (3U1837+04) and 3U1901+03 ($S_{\rm max} \sim 0.3\, S_{\rm crab}$ and $0.1\, S_{\rm crab}$, respectively) make unambiguous detection of a low Aql X-1 flux ($\lesssim 0.2\, S_{\rm crab}$) problematic in the ASM standard resolution mode (see ref. 12 for a complete experiment description). Consequently, occasional fluctuations in detector background and other systematic effects could conceivably result in 'accidental' flux measurements (with low probability) as high as $\sim 0.3\, S_{\rm crab}$ for this particular source. The data obtained during the 1975 and 1976 flares of Aql X-1 are illustrated in Fig. 1, and represent the only unambiguous detections of the source over the ~25 months of ASM operation during which the duty cycle for daily monitoring of the Aquila–Serpens region was >75%. These plots consist of ~½-d averages of the 3–6-keV flux (incident photons) with corresponding ±1σ statistical error bars (estimated 1σ systematic errors are smaller than the statistical uncertainties). The upper limits obtained ($0.1\ {\rm cm}^{-2}\,{\rm s}^{-1}$) immediately before the 1975 flare are consistent with the SAS-3 observations[7], and no positive detections of the source above $\sim 0.2\ {\rm cm}^{-2}\,{\rm s}^{-1}$ were made during the preceding 7½ months. A similar search of the ASM data over the period between the flares yielded no unambiguous source sightings through 1976 April, with a possible detection at $\sim 0.3\ {\rm cm}^{-2}\,{\rm s}^{-1}$ (for ~1 d) 2 weeks before the 1976 outburst. We note finally that the apparently differing peak flux levels of the flares are actually consistent with a single limiting value ($S_{\rm max} \approx S_{\rm crab}$), as degradation in the effective 3–6-keV detector efficiency of ~10% between the two flare observations cannot be excluded.

As a 1.3-d modulation has been reported[8] from the 1975 SSI flare data (obtained during the time interval in Fig. 1*a* labelled 'equator'), we have investigated both the 1975 and 1976 ASM data for periods in the range 0.3–3 d. The technique used consisted of separately folding the data over the range of trial periods and noting deviations in the χ^2-period distribution to the hypothesis of source constancy. The most prominent peak in the 1975 ASM data is, indeed, consistent with the SSI result. As the two experiments have mutually exclusive fields-of-view, the ASM result at a level of 3%±1% is an independent measure of the same effect, but cannot be claimed to be an independent detection owing to its marginal statistical significance. Assuming its reality, we can refine the period estimation to 1.28 ± 0.02 d. No such modulation is evident at this period in the 1976 data, however, with statistical errors comparable to those in 1975.

A summary of the totality of the 1971–76 data is displayed in Fig. 2, from which there is a clear indication that the OSO-7 data are consistent with flaring episodes similar to those in 1975–76. As there is no exact period which can be fitted to the four flares for which there is observational evidence, we have derived a mean flare interval of 435 d with an r.m.s. scatter of ~10%, and have indicated a possible history of identical flares which satisfy all of the observations.

The irregularity of the proposed flare cycle has several obvious implications for source models. In particular, Jones *et al.*[13] have recently reported recurrent flares from 3U1630−47 similar to those discussed here, but which exhibited a regular period of $P = 615 \pm 5$ d. Those authors have suggested that the 3U1630−47 flare cycle may represent the orbital period of an eccentric binary system (as has also been suggested for several transient sources[14,15]), but the observed scatter in the presumed Aql X-1 flare period is inconsistent with the relatively precise timing expected from binary phase-related variations in the accretion flow. The same consideration lessens the likelihood of an association of the present behaviour with a Mira Variable-type binary as proposed for the transients 3U1543−47 (ref. 16) and A1118−61 (ref. 18), or with a Her X-1—like precession-governed modulation[18,19] (as opposed to actual flares).

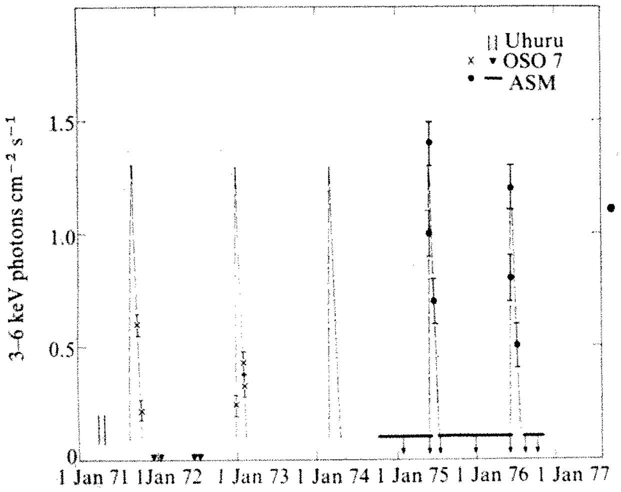


Fig. 2 Summary of Aql X-1 observations over the period 1971–76. The OSO-7 data is taken from Markert[5] and has been approximately converted to ASM units via normalisation with respect to the Crab nebula. Error bars in all data have been conservatively estimated to include possible systematic and normalisation errors. Intermittent Copernicus[6] measurements during 1973–75 (not displayed) are at the level ~0.01–0.02, and do not preclude a 1974 outburst as hypothesised.

We suggest that the observations are most easily interpreted in terms of an 'X-ray dwarf nova' binary of the type proposed by Avni, Fabian, and Pringle[20] for the transient source A0620–00, as the observed X-ray phenomenology is not unlike that of the optical light curves of dwarf novae (and some recurrent novae). Using the empirical period–amplitude relation of Payne-Gaposchkin[21] for U Gem stars and recurrent novae, the inferred luminosity ratio $L_{max}/L_{min} \sim 500$ (assuming that most of the radiation emerges in X rays) yields an expectation value for the interval between flaring episodes of ~465 d. The excellent agreement between the predicted and observed mean flare cycles should not be taken literally, as the uncertainty in L_{max}/L_{min} implies a correspondingly wide dispersion in the expectation flare interval. Furthermore, X-ray dwarf novae may deviate significantly from period–amplitude relations describing their optical analogues[20,22], so we can conclude only that the observed flare interval is not inconsistent with that which might be expected from such a source. The close similarity of the two ASM flare light curves is another model constraint, as it suggests detailed reproduceability. This characteristic of the source behaviour can be reconciled with accretion dwarf nova models invoking relatively continuous mass transfer to an accretion disk which is eventually 'dumped' by an instability[23] or, alternately, quasi-periodic unstable Roche-lobe overflow of the red star[22]. In the former model, the similarity of the flares results from the comparable amounts of material accumulated between episodes, while in the latter a high regularity of the magnitude of Roche-lobe spills and/or a feedback loop (regulated first by self-excited transfer and later by Eddington-limited flow) may be implied. Finally, we note that the non-flaring source behaviour observed by Uhuru (C. Jones, personal communication) may be analogous to the 'standstills' observed in several dwarf novae including Z Cam[23] ($S_{standstill} \sim 0.3$–$0.5\ S_{max}$).

The similarity of the Aql X-1 flares reported here to the more extreme episodes of the transient X-ray sources has been pointed out by several observers[8,24]. The abrupt ($\lesssim$ 1 week) rise by ~2 order of magnitude and subsequent gradual nova-like decay (*e*-folding time ~1 month) back to the pre-flare level are reminiscent of the characteristic transient source light curves, apparently differing only in the degree of brightening (L_{max}/L_{min} for transients is, by definition, $\gtrsim 10^3$) and the absence of a clear precursor peak. This behaviour is, on the other hand, qualitatively different from the recurrent flares of Cyg X-1 (ref. 26) or the extended 'highs' (sometimes lasting for hundreds of days) exhibited by sources such as Cen X-3. In the context of a transient source association for Aql X-1, we note that the relatively soft flare X-ray spectrum[6] suggests a generic relation to the class of 'long-duration' transients[11] (for example, A0620−00 A1524−62, and A1742−28), as opposed to the harder-spectral, shorter-lived transients exemplified by A1118−61 and A0535+26. The identification of the transient A0620−00 with a recurrent nova[26] and the resemblance of the long-term behaviour of Aql X-1 to both the transient X-ray sources and dwarf novae suggests that at least some members of the former group may arise from systems similar to the latter, but with the blue-dwarf binary component replaced by a neutron star or black hole.

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Variability of the BL Lacertae object AO0235+164

WE can summarise previous[1-3] discussion of the problem of excessive Compton scattering in variable compact non-thermal sources by saying that the small size implied by rapid variability leads to too large a probability that a given electron will Compton-scatter one of the emitted photons, leading to an excessive X-ray flux. The basic assumptions underlying these difficulties are that the time-variable radiation is produced by the incoherent synchrotron process by electrons with large pitch angles in randomly oriented magnetic fields, and that the objects are at the cosmological distances implied by their redshifts. We present here a crude model of the recently observed outburst[4] of AO0235+164 in the near infrared and visible, where variability of up to 30% on time scales of 1 or 2 d has been noted. In our model the assumptions of large pitch angle and randomly oriented fields are relaxed[5] and the model accords with our previous discussion[3] of the low-frequency variability of the radio sources 3C454.3 and CTA 102.

The general picture is that of a strong dipolar magnetic field, with field lines of radius of curvature ρ, which is anchored to a spinning massive object, or 'spinar'. Electrons are accelerated by strong electric fields[6] near the surface of the 'spinar', where $E{\cdot}B \neq 0$. They move freely along the magnetic field lines and acquire large momentum components parallel to B.

The observed 1–10-d variability of AO0235 in the near infrared and visible is then due to a burst of electrons of dimension $l \approx ct_V$, where t_V is the observed variability time scale, passing through a region of the magnetosphere in which the magnetic field is nearly tangent to the line of sight to the observer. The beaming angle θ of the burst is either the pitch angle φ of the electrons or the quantity l/ρ, whichever is greater. We show that for the parameters considered here $\varphi < l/\rho$, so that $\theta \approx l/\rho$. Rieke *et al.*[4] find that if the radiation at and near 10^{13} Hz were isotropic and at a distance given by the redshift 0.852, the luminosity in this spectral region would be 10^{47} erg s^{-1}. However, the small beaming angle θ implies that the total luminosity is actually $L_{IR} \approx 10^{47}\theta^2/4\pi \approx 10^{41}\, t_D{}^2/\rho_Y{}^2$, where ρ_Y is the magnetic field line radius of curvature in light years, and $t_D = (86{,}400)^{-1}\, t_V$ is the variability time scale in days. The synchrotron power output of a single electron is $P_{syn} \approx 2\times10^{-15}B_\perp{}^2\gamma^2$ erg s^{-1}, where $B_\perp = B\sin\varphi \approx B\varphi$, in gauss, and γ is the Lorentz factor of the electrons. If N is the relativistic particle density, we have $L_{IR} \approx Nl^3P_{syn}$, and therefore

$$N\gamma^2B_\perp{}^2 \approx 1.5\times10^9\, t_D{}^{-1}\,\rho_Y{}^{-2} \tag{1}$$

The synchrotron lifetime t_{syn} of the particles must be long enough for us to stand a reasonable chance of seeing them. With $t_{syn} \approx 20/(B_\perp{}^2\gamma)$ in yr, $t_{syn} > \rho_Y$ implies

$$B_\perp{}^2\gamma < 20/\rho_Y \tag{2}$$

Also, the electrons must radiate at predominantly 10^{13} Hz. Since we are interested in small pitch angles, we must remember that the usual expression for the 'critical frequency' ν_c for synchrotron radiation holds rigorously only for $\gamma\varphi \gg 1$. But for our purposes, it sufficies to set $\nu_c \approx 6\times10^6B_\perp\gamma^2$ Hz for $\gamma\varphi > 1$. The very small pitch angle case ($\gamma\varphi < 1$) seems to be ruled out because of the high linear polarisations observed[4], which are of the order of 10%. We set $\nu_c = 10^{13}$ Hz to obtain

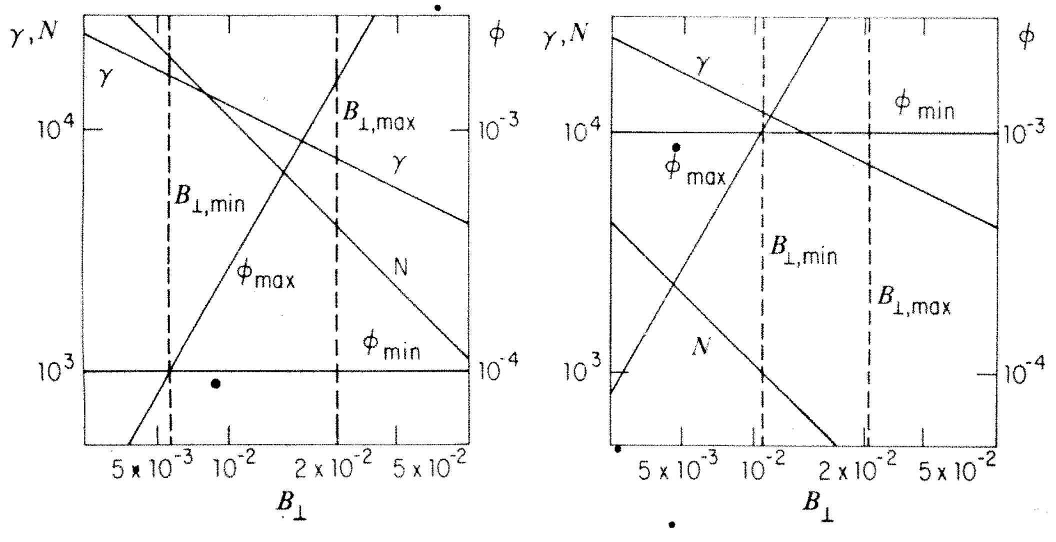
$$B_\perp\gamma^2 \approx 1.6\times10^6 \tag{3}$$

According to Epstein, small[7] pitch angles give linear polarisations $P \lesssim \theta_R/\theta_B$, where θ_R is the angular width of the radiation about the magnetic field lines, so that $\theta_R \approx \varphi + \gamma^{-1}$, and θ_B is a measure of the angular dispersion of the field direction in the source. But θ_B is just our 'beaming angle,' and so we have $P \lesssim (\varphi+\gamma^{-1})/(l/\rho) \approx 300\varphi\rho_Y/t_D$, since $\gamma\varphi > 1$. The observed $P \approx 0.1$ then implies

$$\varphi > 3\times10^{-4}\, t_D/\rho_Y \tag{4}$$

We must now address the problem of excessive Compton-scattered X-ray flux, wherein we require the Compton-scattered X-ray luminosity L_{XR} to be less than L_{IR}. This is more restrictive than requiring the source to be undetected by Uhuru. We are aided by the fact[3,5] that if ψ is the angle between the initial momenta of the electron and photon, the final scattered photon frequency is $\nu_{XR} \approx 2\,\nu_{IR}\,\gamma^2\sin^2(\tfrac{1}{2}\psi)$, and one can show[3] that $L_{IR} > L_{XR}$ implies $1/8N\sigma_T\, l\gamma^2\psi^4 < 1$, for small ψ, where $\sigma_T \approx 6\times10^{-25}$ cm^2 is the Thompson cross section. ψ may be estimated by assuming that the field lines are concentric circles and then demonstrating geometrically that an average ψ is given by $\cos\psi = \rho\cos\varphi/(\rho+1/2l)$, or $\psi^2 \approx \varphi^2 + l/\rho$. But

Fig. 1 The parameters of the model of AO0235+164 variable with a timescale of 1 d (left) and 10 d (right) for a magnetosphere of the size of 1 pc ($\rho_Y = 3$).



since, as we have said, $\varphi < l/\rho$, it follows that $\psi^2 \approx l/\rho$, and therefore the Compton-scattering condition becomes

$$N\gamma^2 < 5\times10^{14}\rho_Y^{2}t_D^{-2} \qquad (5)$$

There is one final constraint that we might impose. Since we wish the general shape of the magnetosphere to remain intact, we require that the magnetic field energy density be greater than the relativistic electron energy density in the burst, or $B^2/4\pi > N\gamma mc^2$. This gives

$$B^2 > 10^{-8}\,N\gamma \qquad (6)$$

The relations (1)–(6) represent six constraints on the five unknowns $B_\perp$, φ, N, γ and ρ_Y (t_D is an observed quantity). From these conditions we obtain the following restrictions on the magnitudes of the parameters $B_\perp$, φ, N and γ as functions of ρ_Y and t_D:

$$1.8\times10^{-4}\,t_D\rho_Y^{-2} < B_\perp < 6\times10^{-2}\,\rho_Y^{-2/3} \qquad (7, 8)$$

$$3\times10^{-4}\,t_D\rho_Y^{-1} < \varphi < 1/3t_D^{1/2}\,\rho_Y B^{7/4} \qquad (9, 10)$$

$$1.7\times10^{4}\,t_D^{-1}\rho_Y^{-4/3} < N < 1.7\times10^{6}\,t_D^{-2} \qquad (11, 12)$$

$$5\times10^{3}\,\rho_Y^{1/3} < \gamma < 3\times10^{4}\,t_D^{-1/2}\,\rho_Y \qquad (13, 14)$$

The inequalities (7) and (8) are obtained from (5) and (2), respectively, by using (3) to express $\gamma \approx 1.3\times10^3 B_\perp^{-1/2}$ and then substituting into (1) to get $N \approx 10^3 t_D^{-1}\rho_Y^{-2}B_\perp^{-1}$. The inequality (9) is just (4), and (10) follows by writing (6) as $\varphi = B_\perp/B < B_\perp(10^{-8}N\gamma)^{-1/2}$ and then substituting for N and γ. The inequalities (7, 8) and (9, 10) are necessary and sufficient conditions for the validity of the model. We have left ρ_Y as an 'external' parameter, since we wish to choose a value for it which is not much different from that in the authors' model[3] for 3C454.3 and CTA 102, where $\rho_Y = 1$.

Careful focus on a typical situation ($\rho_Y = 3$) is depicted in Fig. 1, where the various parameters are presented now as functions of $B_\perp$. The allowed ranges of $B_\perp$ are indicated by dashed lines. Solid lines show the corresponding values of N and γ, and the ranges of the pitch angles φ. The model 'preconditions' $\gamma\varphi > 1$ and $\varphi < l/\rho \approx 3\times10^{-3}t_D/\rho_Y$ are satisfied here.

Thus we see that our crude model, with $\rho_Y = 3$ typically, provides a description of the observed outbursts of AO0235 in the near infrared and visible, with total magnetic fields $B = B_\perp/\varphi$ between 10 and 200 G, electron pitch angles between 10^{-4} and 10^{-3}, and Lorentz factors of 10^4. The magnetosphere is thus about 1 pc in size, which gives total magnetic field energies $B^2\rho^3$ of 3×10^{57}–10^{60} erg, or between 10^3 and 3×10^5 solar rest masses. Total burst energies $N\gamma mc^2l^3$ run between 3×10^{47} and 10^{51} erg.

Since the beaming angle is only 1–10 mrad, we would observe only about one event out of every 1,000. Correspondingly, outbursts of this sort are not often observed. A rough upper limit to the total output of AO0235 can be made by assuming that we observe one event every 10 yr, with a real rate of 100 yr^{-1}. If each outburst has an energy of 10^{49} erg, the average output is 3×10^{43} erg s^{-1}, which is a typical quasar radio luminosity.

The question arises whether synchrotron radiation losses near the surface of the spinar, where the magnetic is much stronger, can substantially exceed the radiated energy in the outer magnetosphere, given by equation (1). A cursory calculation indicates that this is no problem, as long as the magnetic field does not exceed 10^6 G at the surface, taken to be 3×10^{16} cm from the centre[5]. If the field is not accurately dipolar near the surface, but is proportional to r^{-1} or r^{-2} there, there is no conflict with our choice of $\rho_Y = 3$. However, if the field is accurately dipolar, then a model with $\rho_Y = 0.3$ can be shown to be satisfactory.

With such a highly anisotropic electron distribution in pitch angle, there might be problems with instabilities caused by generation of Alfvén waves in a thermal background plasma. This might very well have been the case in the early evolutionary stages of the magnetosphere. The coupling between the Alfvén waves and the beams of relativistic electrons would, on the other hand, mean that the thermal plasma would pick up momentum from the beams, and eventually be swept out of the magnetosphere. After a sufficiently long time, the magnetosphere would be practically evacuated and no further significant Alfvén wave production could occur. Our paper is concerned only with this latter phase.

Our model could also resolve the problem of the low-frequency variability of such radio sources as 3C454.3 and CTA 102, although coherent particle bunching is required. Cocke and Pacholczyk[3] used $\rho_Y = 1$ for these sources. Equally important, Sanders[7] has shown that this general picture is capable of explaining in a natural way the apparent superrelativistic separation of the components of multiple radio sources, such as revealed by recent observations of 3C345 (ref. 9). Small pitch angles are necessary to Sander's discussion also, and expansion along the same direction in successive outbursts is provided by the stable orientation of magnetic axis.

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Uranus occults SAO158687

ON March 10, 1977, Uranus will occult SAO158687, a star of late spectral type with a visual magnitude of ≈ -9.5 (refs 1,2). The occultation will be visible from land areas surrounding the Indian Ocean[1,2]. From high-quality light curves of this event one can obtain temperature, pressure and number density profiles of the uranian atmosphere; information about its composition; and by combining occultation timings from several stations the precise diameter and oblateness of the planet can be found. For the atmospheres of Jupiter[3–5] and Mars[6–9] these quantities have been obtained from recent stellar occultations by these planets. This experience can be used to predict what can be learned from the forthcoming occultation by Uranus.

Although Uranus is several magnitudes brighter than SAO 158687 at visual wavelengths, the strong methane absorption bands in the far red and infrared spectrum of Uranus make the intensity of both objects nearly equal at these wavelengths[3]. To see how to use the methane absorption bands to best advantage, we obtained the spectra of Uranus and SAO 158687 (Fig. 1). These spectra are meant to show the relative instrumental intensities of the two objects and have therefore not been calibrated on an absolute scale. The resolution of the spectra is 10 Å and the cutoff near 9,000 Å is due to a cutoff in

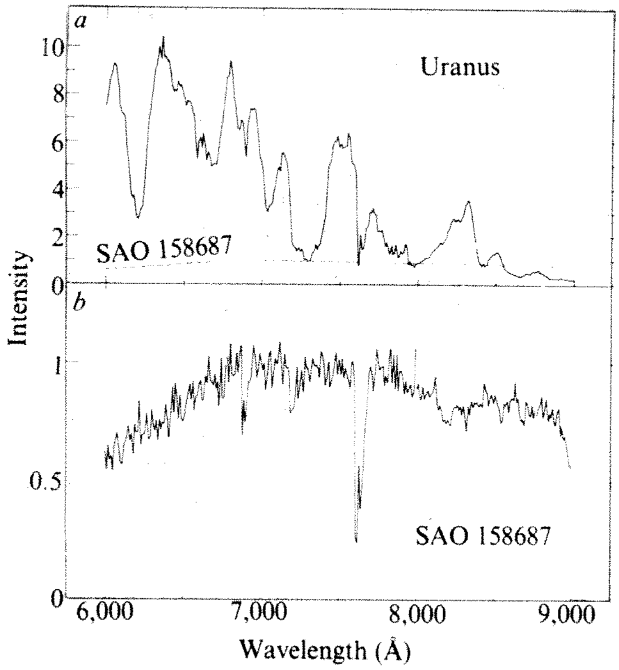

Fig. 1 Relative intensity of Uranus and SAO158687. Each spectrum was obtained with a 10-Å slit; the intensity scales are linear in the instrumental system. *a*, The two spectra on a common scale; *b*, an expanded version of the SAO158687 spectrum. In the deep methane band near 8,600 Å the stellar flux exceeds that of Uranus. The common absorption feature near 7,600 Å is the telluric Fraunhofer A band of molecular oxygen.

the instrumental response. The contribution from the sky background and dark current is negligible.

Table 1 lists four passbands where the relative intensities (integrated over the passband) of SAO158687 and Uranus are most nearly equal. Since the passbands are narrow, the ratio of these intensities is, for practical purposes, equal to the ratio of the photon fluxes f_*/f_u, where f_* and f_u are the photons cm^{-2} s^{-1} Å^{-1} incident on the Earth's atmosphere from SAO158687 and Uranus, respectively. We have assumed SAO158687 to have UBVRI colours appropriate for a K5 main sequence star[1,2,10] and computed the expected photon fluxes f_* (Table 1).

If observing conditions are good during the occultation, then the signal–noise ratio of the light curves should be limited by photon statistics. The latter give a fundamental limit for the signal–noise ratio achievable. We define $\varphi(t)$ to be the flux from SAO158687 observed during the occultation, normalised to the unocculted intensity of the star. Hence $\varphi(t)$ begins at 1.0 and decreases to 0.0 as the occultation proceeds. If ε_φ is the r.m.s. error in φ due to photon statistics for a 1-s integration, then ε_φ is equal to the square-root of the total photons detected from Uranus and the star, divided by the photons that would have been detected from the unocculted star

$$\varepsilon_\varphi = \frac{(\bar{\varphi} f_* + f_u)^{1/2}}{f_* \sqrt{(q \Delta\lambda A)}} \qquad (1)$$

where $\bar{\varphi}$ is the average value of $\varphi(t)$ during a 1-s interval, A is the area (cm^2) of the telescope, $\Delta\lambda$ the passband of the filter (in Å) and q the fraction of the photons incident on the Earth's atmosphere that are ultimately detected by the photomultiplier. The factor q includes the transmission of the Earth's atmosphere, telescope optics, photometer optics and the quantum efficiency of the photomultiplier. The quantity ε_φ has the dimensions of s$^{1/2}$, the actual r.m.s. error in φ for an arbitrary integration time of Δt s is found by dividing ε_φ by $(\Delta t)^{1/2}$. In writing equation (1) we assume that the background counting rates from the photomultiplier itself and the sky are negligible. This may not be entirely true, since a quarter Moon will be only 17° away[1] during the occultation.

Values of ε_φ (Table 1) were computed for $\Delta t = 1$ s, $q = 0.04$, $A = 6.6 \times 10^3$ cm^2 (36-inch telescope) and $\bar{\varphi} = 1/3$.

From Table 1 we see that low photon noise errors can be achieved by choosing appropriate passbands for observation. Such low errors could be realised in ideal conditions, but in practice changes in transparency, guiding errors, severe scintillation[12] and other problems could increase the effective noise in the signal above the level of photon noise.

From the photon noise levels in Table 1, we can calculate the r.m.s. error expected for the temperature profiles obtained from the occultation light curves, if we assume that the gravity gradient model[3] applies to the uranian atmosphere. From the improved procedure of French *et al.*[11] for obtaining temperature profiles from occultation light curves, the minimum r.m.s. error in the scale height $\sigma(H)$, expressed as a fraction of the scale height H is

$$\left.\frac{\sigma(H)}{H}\right|_{\min} \approx 1.5 \left(\frac{v_\perp}{H}\right)^{1/2} \varepsilon_\varphi \qquad (2)$$

where $v_\perp$ is the apparent velocity of SAO158687 perpendicular to the limb of Uranus. The scale height is related to the temperature T by $H = RT/\mu g$, where g is the gravitational acceleration, R the universal gas constant and μ the mean molecular weight of the atmosphere. At the occultation level we expect $T \sim 140$ K (ref. 13), hence $H \sim 65$ km (for $\mu \sim 2$). For this occultation $v_\perp \sim 12$ km s^{-1} (ref. 2). Even for the largest value of ε_φ in Table 1 (0.04), the fractional error in the scale height is only 2.6% (equation (2)). The minimum error in the temperature then will probably be dominated by uncertainties in the mean molecular weight.

At the occultation level the main constituents are likely to be H_2, He and CH_4. For solar composition the mixing ratio of CH_4 would be $\sim 7 \times 10^{-4}$ (ref. 13), which would leave H_2 and He as the major constituents. It may be possible to obtain a value for the helium number fraction [He]/[H_2], from light curves at two wavelengths (6,200 and 8,600 Å, for example), as was done for Jupiter[14], and for the [Ar]/[CO_2] fraction in the case of Mars[9]. *A priori*, it is difficult to predict the expected accuracy of this measurement, since the accuracy will depend on the number and intensity of the 'spikes' in the light curves[3].

The level of the atmosphere probed by the occultation corresponds to a number density n given approximately by the number density at 'half-light' for an ideal isothermal atmosphere[15]

$$n = \frac{\mathscr{L}}{\nu_{STP}} \left(\frac{T}{273\text{ K}}\right)\left(\frac{H}{D}\right) \frac{1}{\sqrt{2\pi (R_p/H)}} \approx 6 \times 10^{13} \qquad (3)$$

where $\mathscr{L}$ is Loschmidt's number, ν_{STP} the refractivity of the

Table 1 Photon noise errors for selected passbands

Centre wavelength (Å)	Passband, Δλ (Å)	Flux ratio, f_*/f_u	Flux† from SAO158687, f_*	r.m.s. photon noise error for a 1-s integration ε_φ
6,200	100	0.14	0.2	0.04
7,300	200	0.5	0.4	0.011
8,000	300	0.7	0.5	0.007
8,600	400	1.2	0.6	0.006

†Photons cm^{-2} s^{-1} Å^{-1} outside the Earth's atmosphere.

atmosphere at STP, D the distance to Uranus and R_p the radius of Uranus.

The 'central flash', observed during the occultation of ε Gem by Mars[16], and used to determine the extinction of the martian atmosphere[9], will probably not be observable, since the centre of the Uranus shadow is predicted to be off the edge of the Earth.

Observers of this occultation should record their data digitally. The shortest timescale of importance for the 'spikes' will be $\sim \delta D/2v_1 \approx 0.3$ s, where $\delta = 5.8 \times 10^{-4}$ arc s (ref. 2) is the angular diameter of SAO158687. Higher time resolution data recording would be desirable, but is not essential.

We plan to observe this occultation from Perth and over the Indian Ocean with the 91-cm airborne telescope[16]. Observers of this occultation interested in coordinating the analysis of their results should write to one of us. We acknowledge the support of grants from NASA.

Note added in proof: An updated prediction for this occultation can be found elsewhere[17].

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Predicted and observed coronal structure

THE total eclipse of the Sun of October 23, 1976 was observed in excellent conditions from Victoria (Australia). The observation programme was designed to study the shape and structure of the corona; this structure is caused by magnetic fields. The corona's shape generally undergoes changes that follow the 11-yr cycle of solar activity. At the time of the eclipse the Sun's activity was near its minimum. In July the mean sunspot relative number was 2.1, in August 16.9, in September 13.4, in October 21.8, and in November 5.5. In these months the activity of the old cycle was still stronger than that of the new one, except in October. The high number of that month was produced mainly by the new cycle. It is not yet certain whether the minimum had been reached by the time of the eclipse. In any case, solar activity was low, but stronger than during most of the earlier minima. Therefore a typical 'minimum-corona' was to be expected, but not the shape of the 'undisturbed corona'. This shape is seldom observed, and was last seen on June 30, 1954, when the Sun was almost completely free of spots for three months before the eclipse[1].

The corona of October 23, 1976, consisted of two main parts completely different in character—a belt of large streamers, going up to heliographic latitudes of $b = 60°$, and the polar regions. As well as some weaker features, there were four large, broad and bright streamers, one in each of the four quadrants. Prominences were found at the base of these streamers, at $b = +41°$ and $-35°$ on the E-limb and at $b = -43°$ on the W-limb. The least developed of the four main streamers was that of the NW-quadrant, in which no prominence was observed. The axes of the streamers deviated strongly from the radial direction and were more or less parallel to the Sun's equator. On the poleside the streamers had a sharp edge. The heliographic latitude of that edge, measured at the Sun's limb, was $+64°$ in the NE-quadrant, $-55°$ in the SE-quadrant, $-60°$ in the SW-quadrant and $+46°$ in the NW-quadrant.

The polar regions were less bright than the main streamers. Each of the two polar cups contained a bundle of about 20 short and narrow streamers, the so-called polar plumes. Around the solar axis their direction was radial, becoming more and more inclined as the polar distance increases.

As the shape of the streamers is formed by the magnetic fields, K. H. Schatten attempted to predict the structure of the corona using the photospheric magnetic fields measured at the Hale Observatories (Mt Wilson) between September 13 and October 10. The result, a computer-drawn plot made on October 18 (ref. 2), is compared with the observations in Fig. 1, which is a sketch of the corona's structure based on photographs obtained at the eclipse. Generally the agreement between observed and predicted patterns is satisfactory. One huge equatorial streamer was predicted for both the east and the west limbs of the Sun, as could be expected for an 'undisturbed' corona. In fact, there were two streamers on each limb, one in each hemisphere, representing the 'minimum-type' rather than the 'undisturbed' corona. The dipolar field represents the polar plumes well. The positions at which the edges of the long streamers limit the polar regions were correctly predicted for the NE- and SW-quadrants. In the SE- and SW-quadrants, however, the observed heliographic latitude of the edges was about 20° smaller than the prediction.

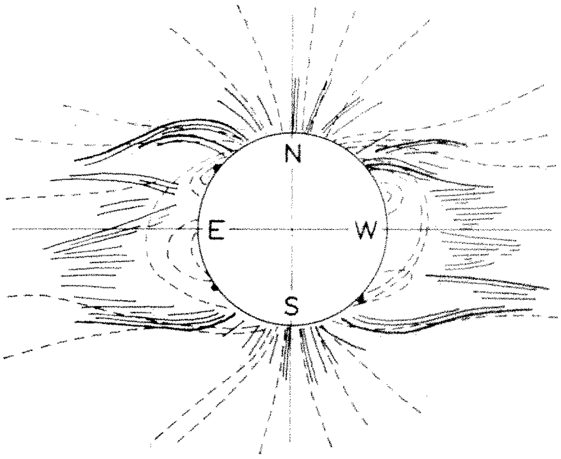

Fig. 1 The structure of the corona at the solar eclipse of October 23, 1976. Observations are represented by solid lines and predictions by dashed lines.

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Improved and extended theory of the Berthelot tube method of studying liquids under tensile stress

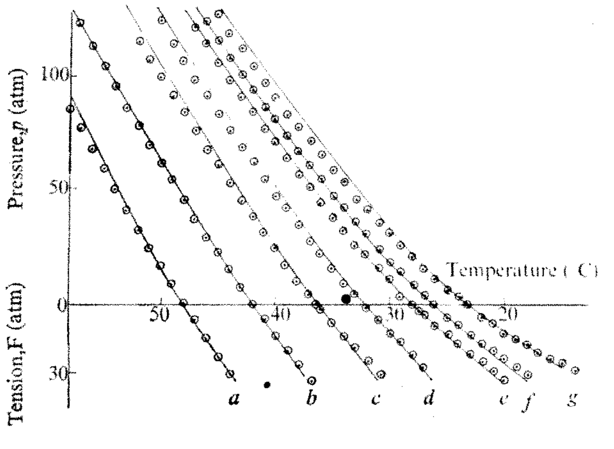
ALTHOUGH the Berthelot tube method of subjecting a liquid to tension has been used intermittently for several years, there is no published theory which enables us to calculate the value of the tension at any stage before the breaking, that is, rupture of the enclosed liquid. We present here such a theory, giving good agreement with the experimental values of the tension obtained directly using a transducer method. In the past, two theories of the method have been given but these only enable us to calculate values of the breaking tension; first, the theory of Vincent and Simmonds (method 1), which we shall show to contain a serious error and second, that of Temperley and Chambers (method 2). These two methods have been discussed by Chapman *et al.*[1].

A liquid can withstand tension due to its ability to exist in a metastable phase. This has applications in work on cavitation, in the design of such things as ship propellers and in the field of lubrication. In its metastable phase a liquid is superheated and this is the basis of the bubble chamber where the path of an ionising particle is shown as a trail of vapour bubbles as it passes through a superheated liquid; the ions produced by the high energy particle act as nuclei on which these bubbles can form. Columns of sap rise to the top of very tall trees due to the existence of tension in the sap column; tensions of > 50 atm have been found in mangrove trees.

The Berthelot tube method is as follows. A sealed tube, usually of glass or steel, is almost completely filled with the test liquid while the remaining volume is occupied by air and liquid vapour. On heating, the liquid expands to fill the whole tube and positive pressures are generated in the liquid. If, next, the tube is allowed to cool slowly, the liquid continues to fill the tube and adhere to its inside walls; during cooling the positive pressure inside decreases to zero and then changes to a negative pressure (tension) until the liquid eventually breaks with the onset of cavitation. At this 'break' there is a sudden increase in the volume of the tube as the internal tension is released. Richards and Trevena[2] have followed these pressure changes directly using a pressure transducer and have obtained some experimental (pressure, temperature, that is) (p,T) curves for water (Fig. 1).

Our proposed theory is as follows. If we consider one of these (p,T) curves (say curve a) then the point on the curve where the pressure is zero corresponds to the state in which both the tube and enclosed liquid are in an unstressed condition. Let V_0 and T_0 be respectively the internal volume of the tube and the temperature at this stage (stage 1). The tube and its contents are then cooled so as to follow the curve to a lower temperature in the tension region where the internal volume of the tube is V_1 and the temperature T_1 ($< T_0$) (stage 2). At this stage tension F is generated in the enclosed liquid and both the liquid and tube are in a state of stress. At this stage we then consider what the unstressed volumes of the liquid and tube would be at the existing temperature T_1. The unstressed volume W_1 of the liquid would be $< V_1$ and the unstressed volume of the tube would be $V_1 - \Delta V_1$ (stage 3). (This is precisely what would happen if the enclosed liquid were to actually break at T_1.) We let ρ_0 and ρ_1 denote the densities of the unstressed liquid at the temperatures T_0 and T_1 (stages 1 and 3, respectively).

Fig. 1 Full lines: experimental (p,T) curves obtained by Richards and Trevena for water in a steel Berthelot tube. ⊙, F and p values calculated using the present theory.



For the unstressed liquid in stages 1 and 3

$$V_0\rho_0 = W_1\rho_1 \qquad (1)$$

Next consider stages 2 and 3 for the liquid at T_1. The volume of the liquid is V_1 under a tension F and W_1 under zero tension ($W_1 < V_1$). Thus, if K_1 is the bulk modulus of the liquid

$$K_1 = -\frac{F}{(V_1 - W_1)/W_1} \qquad (2)$$

Similarly, let us consider stages 2 and 3 for the tube at T_1. Its inside volume is V_1, under an internal tension F, increasing to $V_1 - \Delta V_1$ under zero internal tension, when the liquid breaks. Thus, if K_s is the bulk modulus of the material of the tube,

$$K_s = -\frac{F}{\Delta V_1/(V_1 + \Delta V_1)} \qquad (3)$$

Finally, we consider stages 1 and 3 for the tube; since the tube is unstressed in both cases,

$$V_1 + \Delta V_1 = V_0(1 - 3\alpha\Delta T_1) \qquad (4)$$

where $\Delta T_1 = T_0 - T_1$ and α is the linear coefficient of expansion of the material of the tube.

Equations (1) – (4) give the 'basic physics' of the method and can be solved to give an expression for the tension F

$$F = \frac{\rho_0/\rho_1 - (1-3\alpha\Delta T_1)}{\rho_0/\rho_1 K_1 - (1-3\alpha\Delta T_1)/K_s} \qquad (5)$$

in terms of the known or measurable quantities α, ΔT_1, ρ_0, ρ_1, K_1 and K_s.

Suppose that we return to our initial stage 1 and move up the curve a of Fig. 1 to a point in the pressure region; let the pressure inside the tube at this stage be p and the corresponding temperature T_2 ($> T_0$). We can then obtain a value for the pressure p generated inside the tube at the temperature T_2

$$p = \frac{(1 - 3\alpha\Delta T_2) - \rho_0/\rho_2}{\rho_0/\rho_2 K_1 + (1 + 3\alpha\Delta T_2)/K_s} \qquad (6)$$

where $\Delta T_2 = T_2 - T_0$ and ρ_2 is the density of the unstressed liquid at the temperature T_2.

The present theory enables us to calculate the tension or pressure in the enclosed liquid at any stage of the process, using equations (5) and (6), thus improving on previous theories of the Berthelot method which only give an equation for the value of the actual breaking tension.

From integral values of ΔT_1 and ΔT_2 we can compute the values of F and p from equations (5) and (6) using the appropriate values of the other quantities involved as obtained from tables. These calculated values are shown in Fig. 1 and the agreement between theory and experiment is satisfactory.

In method 1, Vincent and Simmonds write

$$V_b = V_f[1-3\alpha(T_f-T_b)] \qquad (7)$$

where V_f is the volume at the filling temperature T_f and V_b that immediately after the break at the temperature T_b when the tube is in an unstressed state. But an equation such as (7) can only be applied provided the tube is in the same state of stress at both T_f and T_b. Chapman *et al*[1] have, however, shown that pressures of 50–100 atm exist in a Berthelot tube at T_f. Thus the theory of method 1 is incorrect since it only takes into account volume changes due to temperature differences and ignores those due to the change in the state of stress of the tube; our theory takes both of these factors into account. Method 2 predicts values for the breaking tension which agree quite well with the experimental values obtained directly from the transducer measurements of Richards and Trevena. One great advantage, however, of our theory is that equation (5) enables us to calculate the tension inside the tube at any stage before rupture; methods 1 and 2 only enable us to calculate the values of the actual breaking tension (and method 1 does that incorrectly). Also, our theory is in satisfactory agreement with experiment, in both pressure and tension regions (Fig. 1)

We hope that we have corrected some previous errors, and both extended the Berthelot tube theory and put it on a firmer foundation.

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Ion-pair clusters and excited-state yields in radiolysis

WHEN β or γ rays are absorbed in matter, sparse tracks of ion pairs are produced. the secondary electrons sometimes have enough energy to produce further ion pairs in small clusters Distributions of cluster size in a cloud chamber were measured by Wilson as long ago as 1923 (ref 1); reinterpretation[2] of his and other results shows that nearly two thirds of the ion pairs occur in clusters. Similar behaviour should occur in other phases, but little is yet known of the role of clusters in radiolysis In hydrocarbons, the geminate recombination of radical ions is a major process: most theoretical descriptions have dealt only with single ion pairs[4]. Since most of the ion pairs recombine very quickly, the clusters will rapidly become single pairs, so the approximation is probably quite good for scavenging studies. If we consider recombination products, especially the yields of singlet and triplet excited states, cluster formation must be taken into account[4]

Measurements made by pulse radiolysis[5–7] of yields of excited singlet and triplet states, $^1M^*$ and $^3M^*$, in alkanes and benzene derivatives containing added aromatic hydrocarbons (M) have shown triplet-to-singlet ratios, T/S, ranging between 1 and 2. Hereafter 'triplet' and 'singlet' will usually refer to the ion pairs Though the ions are so far apart (~50–100 Å) for most of the life of a pair that the unpaired electrons do not interact, one can still speak of singlet or triplet character[8,9].

A single ion pair will normally be formed in a singlet state if recombination is fast enough, only $^1M^*$ will be produced[8] Single triplet pairs will be formed if the secondary electron has sufficient energy to excite another molecule to a triplet state by electron exchange[4] (process A). In clusters, some triplets will be formed by cross-recombination (process B). Calculation of T/S for B is a surprisingly difficult mathematical problem; however, combining the results of Atkins and Lambert[10] with the spur size distribution[3] gives 0.34.

Initial spin correlation in an ion pair will decay over some tens of nanoseconds[8]; the rate of decay and its final extent can be changed by magnetic fields[8,9]. In the simple case of a single pair, initially singlet, the final state at zero field consists of one quarter singlets, three quarters triplets. in a strong magnetic field, the singlet can only interact with T_0· the T± components become inaccessible because of their Zeeman energy: the final state is half singlet, so the field should increase the yield of $^1M^*$ and of fluorescence by a factor of 2 at long times.

By the single-photon counting method, I measured scintillation pulse shapes for solutions of *para*-terphenyl, anthracene (and their perdeutero-analogues), biphenyl and perylene in a number of alkanes and benzene the enhancement ratios at long times (~100 ns after ionisation) are in the range 1.30–1.45 for alkanes· there is little or no dependence on the nature of the solute. These are tentative figures. the limiting values may not be reached, or the yields of $^1M^*$ and $^3M^*$ may be influenced by energy level densities (Frank–Condon factors)[8]. However, in both cases one would expect to see differences between solutes. Therefore, I propose that these low values are due to scavenging of initially triplet ion pairs. Triplets decay into singlets reducing the net effect; if the initial T/S were 3, there would be no net change and the field effect would disappear: the above range corresponds to initial T/S ratios between 1 1 and 0.7 This interpretation is supported by the case of benzene solutions, where the enhancement is only 1.09 (T/S ~ 2.15). The benzene triplet state lies well below those of the alkanes, so that process A will be more important

Total yields of $^1M^*$ and $^3M^*$ (refs 5–7) are less easy to interpret: the excitation mechanisms are still being debated; in particular it has been proposed[6] that singlet excitation transfer from the solvent is the main source of $^1M^*$ rather than ion recombination[7] Also, ion recombination is slow enough that significant decay of the spin correlation occurs before recombination. The extent of this can be estimated from measurements of enhancement of fluorescence and is about 10% for solutions in cyclohexane[12], that is a measured T/S ratio of yields of 1, would correspond to an initial ratio of 0.82. Precise calculations are not possible at present, but there appears to be no inconsistency between the magnetic effect data and the measurements with pulse radiolysis, provided that recombination is the major source of excited states in alkane solutions

In striking contrast is the work of Lipsky *et al.*[13,14] who have measured the fluorescence yields of the alkanes themselves, avoiding complications produced by solutes. For bicyclohexyl, they estimate that T/S ≤ 0.25; this is an initial value since recombination in the alkanes is extremely fast (picoseconds)

The discrepancy can be explained by noting that processes A and B, in which an overall singlet spur produces two or more triplets, are both reversible; for example, in a two-pair cluster a triplet ion pair may recombine to a triplet molecule, which can exchange spins with the second electron as it returns to the neighbouring cation (being quenched in the process). Scavenging of electrons by a solute will allow more time for cation and triplet or two cations to separate, even though ion recombination may still occur in ≳1 ns. For solutions, the enhancement by the field varies considerably among the alkanes, this can hardly be due to process A; there seems to be a correlation with positive charge mobility—probably an effect of scavenging rate On this basis, one would expect larger overall yields of excited states in benzene etc, and this is found[13,14], process A, however, also contributes to this.

These results demonstrate the usefulness of the magnetic field effect for studying geminate ion recombination· measurements at long times are free from interference from energy transfer, which must be complete in a few nanoseconds; free ions only contribute to the background which is subtracted out.

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Resistivity and the continental crust in southern Africa

DURING the last decade many deep electrical soundings have been made over the major Precambrian tectonic provinces of southern Africa[1,2]. The results (Van Zijl, submitted for publication) show that there are marked differences in the electrical properties between Archaean cratonic terrains (Kaapvaal and Rhodesian cratons) and mobile belts (Limpopo, Namaqua and Damara). The ages of the cratons are >2,600 Myr, whereas the dominant ages of the mobile belts are 2,700, 1,000 and 500 Myr, respectively[3]. In the discussion of crust here, the overburden or cover rocks of the various tectonic provinces have not been considered.

Cratonic terrains are characterised by a highly resistive zone extending from the surface down to a depth of 5–10 km depending on the locality. The average resistivity of this zone is about 100,000 Ω m with values varying between 30,000 and 500,000 Ω m. In some areas the highly resistive layer is overlain by a more conductive layer with an average resistivity of about 55,000 Ω m and a variable thickness.

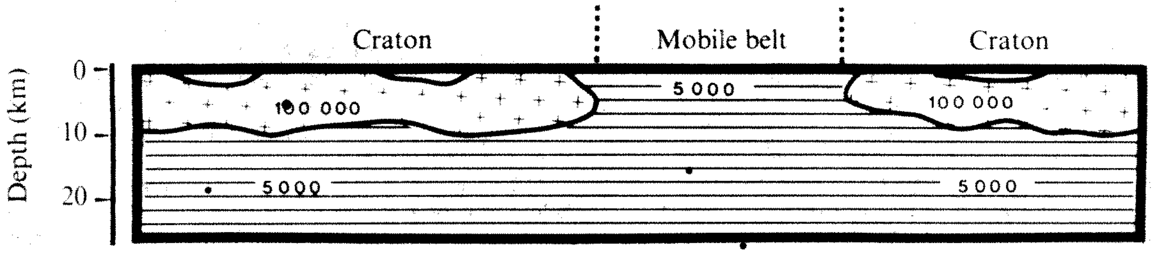
In contrast, the prominent highly resistive zone associated with the cratons is completely absent in the mobile belts. Instead, a thick moderately resistive zone with a resistivity ranging from 2,000 to 8,000 Ω m (average 5,000 Ω m) extending to a depth of 25–30 km is recognised. An important feature of this zone, deduced from closely spaced soundings, is that it continues laterally into the craton where it underlies the highly resistive layer. The electrical model is shown schematically in Fig. 1.

Correlation of the relevant electrical horizons with the results of geological investigations[1-10] shows the following relationships. First, the uppermost 5,000 Ω m layer observed in cratonic areas is due to the presence of metamorphic rocks of supercrustal origin. Second, the highly resistive zone in cratonic terrains is associated with vast amounts of granitoid material in the form of granitic intrusions, gneisses and migmatites. Third, the 5,000-Ω m layer of the mobile belts can be attributed to the presence of intensely deformed and highly metamorphosed rocks exhibiting features of upper amphibolite and granulite facies metamorphism. Fourth, the lower crust is metamorphic as deduced from the continuation of the 5,000-Ω m layer under the highly resistive layer associated with the cratons.

The view that the stable continental crust of cratonic areas is made up of a lower crust of high-grade metamorphic rocks underlying a more granitic upper crust which also contains remnants of supracrustal metamorphics, is not new. It was proposed, on geological grounds, by Wegmann[11] as early as 1935 and is supported by numerous later studies[12-18]. Geophysical arguments based particularly on heat flow[13], seismic[19] and gravity[20] investigations agree with this model. Basically, the lower crust is considered to consist of rocks which have largely been depleted of lighter granitic materials, volatiles and radioactive elements, leaving behind a refractory residuum. The metamorphic layer grades upwards into a zone in the upper crust consisting predominantly of granitic material occurring in the form of intrusions, gneisses and migmatites which were mainly mobilised from melts originating from the lower crust. It has often been noted that rocks of this zone both intrude and form the basement to supracrustal metamorphic rocks[4,14,21]. The extremely high resistivities associated with the granitoids indicate that the rocks are solid, strongly consolidated and largely unfractured.

The suggested correlation of the highly resistive layer with cratonic upper crust is significant as it provides a means of determining the extent of cratonic material even into adjoining mobile belts provided the grade of metamorphism remains low enough to prevent the migration of the granitoid material to higher tectonic levels[11]. Results to date indicate that the distribution of the ancient cratonic layer was indeed extensive and give the impression that this layer was broken up by the mobile belts studied. The Limpopo and Namaqua mobile belts, where a highly resistive cratonic material is bound on both sides of a core of moderately resistive highly metamorphosed rocks, are good examples. The view that the southern African mobile belts represent reworked and reconstituted cratonic crust[8,9,10,11,18] is thus supported by the electrical studies. Further evidence comes from complementary electrical information derived from the results of magnetometer array studies[21,22] which show that these mobile belts are not underlain by a highly conductive lithosphere as would be expected if they represented the traces of subduction zones involving oceanic crust. The mobile belts are thus regarded as being of ensialic origin, as suggested also by geological evidence[8,10].

Fig. 1 Schematic resistivity model of the Precambrian continental crust obtained from direct current Schlumberger electrical soundings. The average resistivities (Ω m) of the electrical horizons are shown. The highly resistive zones are associated with granitoid material (+) in cratonic terrains, whereas the more conductive zone is correlated with intensely deformed high-grade metamorphic rocks (—). The isolated synforms (white) overlying the granitoid material represent supracrustal metamorphics. Cover rocks are not shown.



The resistivity-derived model indicates that the floor of the mobile belts is formed by high-grade metamorphic rocks which are probably of deep crustal origin. In accordance with the views of Kröner[9] and Shackleton[18] it seems that the crust has become decratonised with time and is weakened, not stiffened, by events of deformation and metamorphism which have often been polycyclic.

The electrical model can be extended when account is taken of the absolute values of resistivity obtained from deep sounding results. From available temperature gradient estimates[23], it can safely be assumed that the temperature at a depth of 25–30 km is too low for conduction through minerals to be important[24] and that crustal rocks conduct electricity by virtue of interconnected cracks filled with water[24]. The space occupied by water can be calculated if the rock resistivity and the water resistivity are known[25]. While the latter parameter cannot be measured directly at great depths, it can be estimated by the extrapolation of water resistivities measured near the surface. From measurements on samples from boreholes and in mines (van Zijl, submitted for publication) a value of 1 Ω m has been assumed for water at depth. On this basis the porosity of the upper crustal cratonic layer is <0.5%, which substantiates its basically solid and unfractured nature as noted earlier. The mobile belts, on the other hand, have considerably larger porosities, varying from 3% near the surface to about 1% at a depth of 25 km, implying that these rocks are cracked and fractured and consequently weakened, not only in mobile belt areas but also in the lower crust under the cratons. Various studies have indicated that pore pressure is high during metamorphism and that a high degree of crack space is required[24,28]. These observations considered jointly with the intensely deformed nature of these rocks, may explain the origin of the cracks. The depth to which the connectivity of crack space continues will depend on the temperature at which permanent deformation of the rock minerals occurs. Experimental work[27] has shown that temperatures in excess of 500 °C are needed, which correspond to depths greater than 30 km in normal crustal situations. When viewed over geological periods, the interconnectivity of cracks, which come right up to the surface in mobile belts, implies hydrostatic pore pressure[28] and a decrease of porosity with depth[28]. This supposition is supported by the resistivity results over mobile belts, as noted earlier. The origin for the water in the crust may thus be meteoric[29]. The several-fold increase of the porosity at the boundary of cratonic layer and the underlying metamorphic zone may account for the low velocity layer found at this depth in the Canadian Shield[30], a region geologically similar to the areas studied in southern Africa.

The suggestion that free water is available at all levels in the crust has many implications regarding physical processes and geochemical effects. The variation of heat flux and the nature of the crust–mantle boundary are only two examples.

Thermal gradients[31] and heat flow estimates (A. E. Carte, personal communication) from mobile belts appear to be anomalously high when compared to cratonic terrains, especially when the lower heat production of highly metamorphosed rocks[13] is taken into account. This paradox may possibly be explained by the transport of heat by the preferential upward movement of water through the cracked metamorphic zone and its concentration in mobile belts. Regarding the crust–mantle transition zone, electrical sounding results sometimes show the presence of a conductive layer at this depth which is interpreted at present as being due to hydration processes of mantle rock (van Zijl *et al.*, in preparation).

It is clear that electrical resistivity is intimately associated with both the nature and the structure of the continental crust; its usefulness as a tool in crustal investigation critically depends on the quality of the data obtained from electrical investigations.

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Age and position of the Ellsworth Mountains crustal fragment, Antarctica

The present geographical position of the Ellsworth Mountains is geologically anomalous on account of their stratigraphy, which does not relate to western Antarctica, their structural trend, approximately perpendicular to the trend in adjacent regions, and their relief, the greatest, and possibly the youngest, in Antarctica. These factors suggest that the Ellsworth Mountains are not in their original position but have moved, probably during the Cainozoic.

During a radio-echo ice-depth-sounding flight on January 22, 1975 from the remote American 'Siple' station in inland Antarctica, a British Antarctic Survey aircraft landed beside two small nunataks at the edge of the Ronne Ice Shelf at 77°02′S, 78°20′W (Fig. 1). In 1947, Ronne[1] sighted a nunatak at 77°40′S, 79°00′W which he named Mount Haag. As we found no other nunataks within 180 km, these are considered to be Mount Haag, now renamed Haag Nunataks.

Under the direction of Dr C. W. M. Swithinbank, seven specimens of the *in situ* rock types were collected comprising four granodiorite–gneisses, one hornblende schist, one quartzo-feldspathic gneiss and one acid vein cutting gneiss. All but one of the rocks show acid veining to some extent.

The granodiorite–gneisses generally have a strong foliation marked by biotite (<10%) which is frequently altered to chlorite and associated with hornblende (<3%). Quartz crystals are mostly strained, often fractured and may occur as single crysals or in a mosaic. Oligo-andesine, usually heavily sericitised, is predominant, and potash feldspar is present mainly as microperthite of a string or bead type, but microcline with characteristic twinning also occurs.

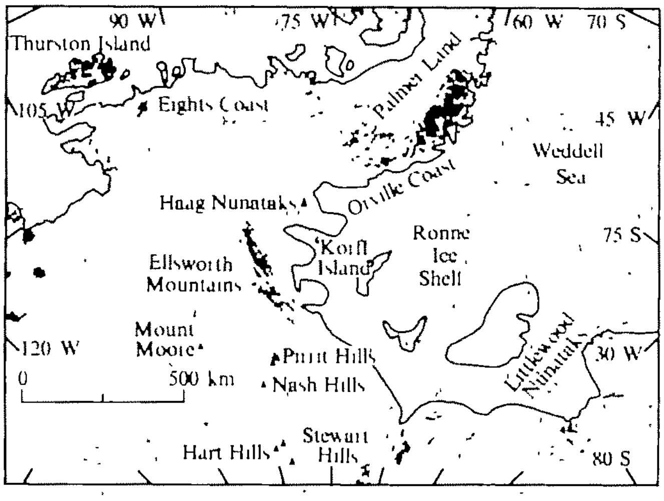

Fig. 1 Map of the Ronne Ice Shelf and its environs; areas of exposed rock are shown in black

Potash feldspars rarely show any alteration, and clear untwinned feldspars are probably cryptoperthite. Accessory minerals include pistacite, sphene, allanite and ore minerals.

The hornblende schist has a granoblastic texture and comprises about 29% green hornblende, 39% andesine with quartz, biotite, accessory sphene and some ore. Acid veinlets and augen in the schist are much richer in quartz and contain some potash feldspar whereas hornblende is a minor constituent. The quartzo-feldspathic gneiss consists of microcline augen, quartz, perthite, plagioclase and biotite. The specimen of an acid vein in gneiss is essentially similar in the vein part to the quartzo-feldspathic gneiss and to the grandodiorite–gneisses in the gneissic part.

These rocks seem to have been regionally metamorphosed to amphibolite facies and suffered synorogenic invasion by acid veins. No observations of field relationships could be made but colour photographs of the outcrop suggest that some of the rocks may be migmatites.

This suite of rocks is typical of many regional metamorphic terrains of Antarctica but the nearest known outcrops of similar rocks are along the Eights Coast and in the eastern part of Thurston Island[2] or in the coastal regions of northern Palmer Land[3] 'Granites intrusive into gneisses' were reported, however, at 81°53′S, 69°20′W by the 'Ellsworth' station–'Byrd' station traverse party[4]. The nearest outcrops inland of the Orville Coast[5] and in the Ellsworth Mountains[6] comprise sedimentary rocks, those in the Nash and Pirrit Hills contain granitic intrusions, and Mount Moore and the Stewart Hills consist of phyllite and schist[7]. Behrendt[8] reported a nunatak to the west of 'Korff Island' but this is now considered to be an ice fall (C. W. M. Swithinbank, personal communication).

K–Ar determinations on two gneisses from Haag Nunataks have yielded the results shown in Table 1.

The concordant dates obtained from separates of hornblende and co-existing biotite provide a minimum age for the last major regional metamorphism affecting the gneisses. The lower apparent ages obtained from the potash feldspars and plagioclase are due to argon loss from these less retentive minerals, and are not necessarily geologically significant. It is interesting that a date of 1,044 Myr (ref. 9) has been obtained from rhyolites in the Littlewood Nunataks and may reflect some association in time and space with the gneisses from Haag Nunataks.

Schopf[10] suggested that the Ellsworth Mountains lie on a fragment of continental crust which has moved to its present position from the north-east. Assuming Haag Nunataks are an up-faulted slice of the metamorphic basement underlying the Ellsworth Mountains, these dates provide additional evidence to suggest that the Ellsworth Mountains area was originally along the western margin of the Antarctic plate. Apart from a deep subglacial trough between the Ellsworth Mountains and the Orville Coast[11] which may continue eastward to the north of Haag Nunataks, there are insufficient data to delimit the margins of this fragment of continental crust until the bedrock topography of the Weddell Sea and its environs is known. The structure and stratigraphy of the Ellsworth Mountains[11] and the ages from Haag Nunataks suggest an original position for this fragment of continental crust westward of the Littlewood Nunataks (where there is a bathymetric depression), but an accurate palaeogeographical reconstruction of this region must await palaeomagnetic data from the Weddell Sea floor.

Table 1 Analytical data

Sample no.	Material analysed	% K	Vol. rad. ^{40}Ar (nl g^{-1})	Age (Myr)
E.4690.1	Hornblende	1.04	55.79	1,018±28
	Biotite	6.39	332 3	991±22
	K-feldspar	10.4	381.5	745±18
	Plagioclase	0.553	16 45	628±18
E.4690.2	Hornblende	1.01	55.30	1,031±14
	Biotite	6.79	358.3	1,002±24
	K-feldspar	9.99	356.2	731±18
	Plagioclase	0.676	18.86	595±16

Ages have been calculated with $\lambda_\beta = 4.72\times10^{-10}$ yr^{-1}, $\lambda_e = 0.584\times10^{-10}$ yr^{-1} and ^{40}K/K = 0.0119 at %.

Errors are quoted at the 2σ level and take into account uncertainties in the mass spectrometer ratio measurements, spike calibration, atmospheric argon correction and potassium determinations.

Argon concentration was determined by standard isotope dilution techniques and potassium was determined in quadruplicate by flame photometry.

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Geochronological evidence of Hercynian activity in Newfoundland

A RECONSTRUCTION of the continental blocks for the central and North Atlantic has led to the widespread acceptance of the continuity of the Palaeozoic orogenic belts on both sides of the Atlantic. A reconstruction of the land masses during the Carboniferous and Permian shows the Variscan Belt of Europe, the Mauritanides of Morocco, and the

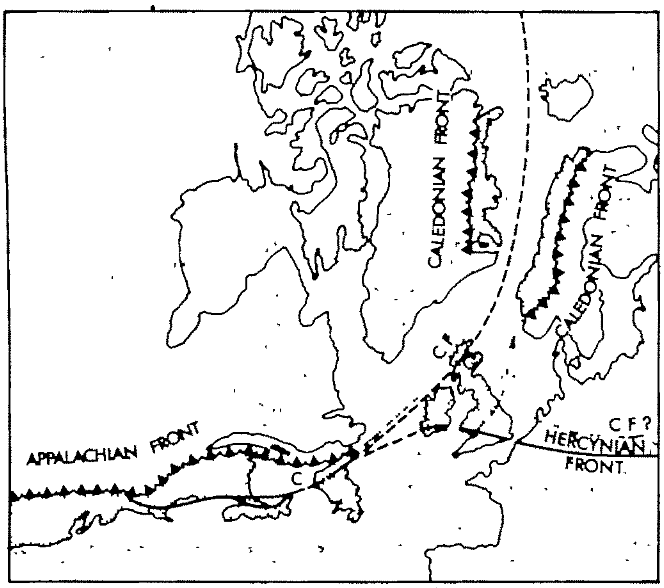

Fig. 1 Reconstruction of continental blocks around the North Atlantic during the Permian (after Holmes[3]) C. F., Caledonian Front of northern Scotland; G, Great Glen Fault; C, Cabot Fault

Southern Appalachians as being continuous[1]. Although the Hercynian Front has now been recognised in New Brunswick[2] the situation in Newfoundland is unclear We suggest, on the basis of the geochemical data outlined in Table 1, that the continuation of these belts can be interpolated between New Brunswick and south-west England to include at least the Central Mobile Belt and the Avalon Zone of Newfoundland We find the earlier correlation by Holmes[3] particularly attractive in view of our new isotopic data, a correlation which extends the Hercynian Front westwars from south-west England and Wales into Canada, where it passes along the Cabot Fault into the New Brunswick seaboard and the Bay of Fundy (Fig. 1) Cherkis *et al*[4] link the Charlie Gibbs Fracture Zone to the Hercynian Front on both the European and North American continents, and have recently offered new evidence for the emergence of the front in Newfoundland[5]. The intersection of the Charlie Gibbs Fracture Zone and the continental shelf of Newfoundland lies about 100 km east of the Cabot Fault, so that their delineation of the front lies further to the south than the one outlined here

The four granites listed in Table 1 and shown in Fig 2 record either intrusive or deformational events of Carboniferous age. Three of these granites are emplaced into the crystalline margins of the Central Mobile Belt, while the St Lawrence granite is intrusive into Palaeozoic and Late Precambrian sedimentary and volcanic sequences of the Avalon Zone. The St Lawrence granite, a non-foliated, high-level, peralkaline body associated with fluorite mineralisation, intrudes a zone of weakness marked by a series of north–south trending folds, north–east trending synclines, and high-angle thrusts that run parallel to the fold axes[6]. The remainder of the granites are either sheared or cataclastically affected by regional deformation. The Lockers Bay granite, a megacrystic microcline variety, is intimately associated with the Dover Fault, a major tectonic discontinuity that separates the northern part of the Central Mobile Belt from the Avalon Zone. This granite was considered to be Hadrynian in age, or older[7]. To the north, in the Burlington Peninsula area, the regional deformation which post-dates the intrusion of the LaScie intrusive complex and the Cape Brulé porphyry has been attributed to either pre-Ordovician[8,9] or post-Lower Ordovician–Devonian movements[10]. At least two phases of deformation have affected the LaScie complex; using some of the geochronological evidence presented here, DeGrace *et al*[11] concluded they "are probably both Hercynian deformations"

Widespread basement reactivation in Europe during late Carboniferous times (Westphalian–Stephanian) produced many of the granitic masses of the Bohemian Massif, the Iberian Peninsula, Morocco, the Schwarzwald and the Massif Central. As a consequence of partial melting of a thickened continental crust (brought about by continent–continent collision of the northern and southern European plates) these granites were enriched in potassium relative to their more sodic counterparts formed during the Caledonian Orogeny[12]. Similarly, the granites listed in Table 1 seem to be quite different to granites of Devonian age that we have dated from Newfoundland. The marked contrast of the initial ratios shown in Table 1 to the lower ratios (0.704–0.709) for many of the Acadian granites is entirely consistent with derivation of the younger granites by partial melting of continental crust.

Variations in age, deformation and metamorphism along the length of the Caledonian–Appalachian–Hercynian

Table 1 Whole-rock Rb–Sr isochron data*

Locality	Petrographic and geochemical notes	Apparent age (Myr)	Initial $^{87}Sr/^{86}Sr$ ratio	No. of points
LaScie Intrusive Complex	Fine- to medium-grained alkali granite; mainly quartz, orthoclase; rare plagioclase, amphibole and opaques; traces of epidote and carbonate—evidence for cataclasis	315±25	0.715±0.012	5
Cape Brulé porphyry	Feldspar and embayed quartz phenocrysts set in fine-grained groundmass; alteration products, sericite, chlorite; traces of calcite, fluorite; some evidence for cataclasis	325±14	0.7145±0.0018	5
Lockers Bay granite	Megacrystic microcline, biotite granite; strongly schistose —microcline phenocrysts some flattened; granulation of quartz and feldspars; epidote common	300±18	0.7146±0.0013	6
St Lawrence granite	Red to pink, medium- to fine-grained; mainly quartz, orthoclase, albite, with minor riebeckite, aegerine, biotite, fluorite, magnetite and haematite, perthitic and granophyric textures common	315±10	0.722±0.005	4

*$\lambda^{87}Rb = 1.47 \times 10^{-11}$ yr^{-1}.
All errors given at the 2σ level.

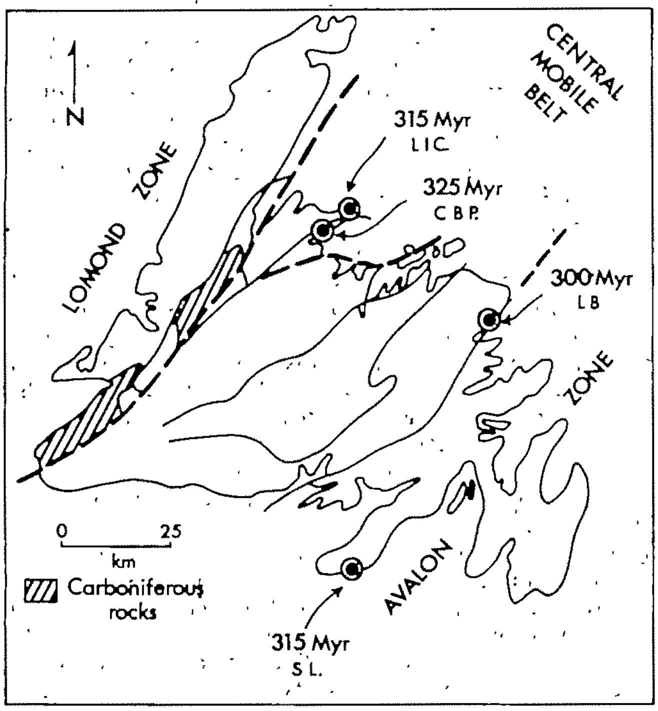

Fig. 2 Distribution of known Carboniferous granites in Newfoundland. L. I. C., LaScie Intrusive Complex; C. B. P., Cape Brulé porphyry; L. B., Lockers Bay granite; S. L., St Lawrence granite.

orogen have been attributed to either irregularities in the geometry of the colliding continental plate margins[11], or to an oblique collision[14]. The result, in both cases, is the formation of numerous small plates, wedges and splinters of continental crust, usually bounded by transcurrent faults. After the Hercynian orogeny the division of Newfoundland into a series of independent fault-bounded blocks could account for the distribution and origin of many of the geological features that are now observed in the Central Mobile Belt High-angle faults, many of them transcurrent, abound in the central part of Newfoundland, and are especially numerous close to the boundary between the Central Mobile Belt and the Lomond Zone. Some time after collision, crustal thickening along with transcurrent faulting led to partial melting of the continental crust which produced Mid-Carboniferous magmatism and mineralisation Deformed Carboniferous strata contained in a narrow, elongate, fault-bounded trough which trends from Cape Anguille in the south-west to White Bay in the north-east gives some stratigraphic significance to the interpretation of our isotopic data. The unconformable relationship of the Deer Lake Group with the basement and the Anguille beds in north-central Newfoundland testify to early and late Mississippian deformation[15].

Both the stratigraphic and isotopic evidence support the model of Dewey and Burke[1], which includes southern Europe colliding with Europe/North America in Mid-Carboniferous times The later post-Stephanian (Maritime or Alleghenian) disturbance further to the south coincided with Africa south of the South Atlas Fault sliding westward to collide with North America[16].

For convenience, and to follow earlier workers, we use the term 'Hercynian Front' here, even though its meaning is poorly defined

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Slow recolonisation of deep-sea sediment

USING the submarine ALVIN, experiments on deep-sea benthic communities have been designed to follow the recolonisation of defaunated deep-sea sediments and to provide estimates of growth rates and time to maturity in individual species Small brood size and the high proportion of adults in a number of deep-sea benthic taxa suggest relatively low rates of recruitment, growth and mortality in the deep sea[1]. Large individuals (8 4 mm) of the deep-sea bivalve, *Tindaria callistiformis*, have been estimated to be at least 100 yr old[2]. Measurements of microbial activity and community respiration indicate rates one to three orders of magnitude lower than in shallow water[3,4]. In contrast with the view that there is a general slowing of life processes, the first *in situ* deep-sea measurements on molluscan borers in wood panels have indicated rapid rates of recruitment, growth and maturation. Wood is, however, an ephemeral resource and its occurrence in the deep sea is unpredictable, while the majority of deep-sea habitats are thought to be highly predictable, therefore favouring less opportunistic life histories.

Studying community succession in defaunated sediments gives hope of predicting the rate of recovery of deep-sea communities following a disturbance and assessing the normal role of disturbance in structuring these remarkably diverse communities. The *in situ* placement of defaunated sediments in benthic studies is similar to the use of fouling panels or artificial three-dimensional habitats

In September 1972, the research submersible ALVIN placed boxes (50×50×10 cm) of azoic sediment at a depth of 1760 m at the first Woods Hole Oceanographic Institution Permanent Bottom Station (PBS 1) (39°46′N, 70°40′W). The sediment was previously collected in the same area at approximately the same depth with an anchor dredge. It

Table 1 Results of experimental boxes of azoic sediment placed and recovered in shallow water and the deep sea

Station		Time exposed (months)	Density of individuals (m^{-2})	No of species	No. of individuals	Sample area (m^2)
Buzzards Bay	Control	—	48,375	61	1,935	0 040
(10 m)	Expt	2	35,714	47	704	0.021
PBS 1	Control	—	5,189	> 103	454	0.0875
(1,760 m)	Expt 1	2	160	14	43	0.250
	Expt 2a	26	564	31	141	0 250
	Expt 2b	26	536	10	15	0.028

Table 2 Species contributing more than 2.3% of the individuals in the 2-month and 26-month experimental boxes and 25 control cores

25 Control cores		26-month box PBS 1		2-month box PBS 1 (1 individual is 2.3%)	
Prochaetoderma sp.	7.5%	Gnathiid isopod	13.5%	*Priapulus atlantisi*	30.2%
Spionid (undescribed)	5.1%	*Neopodarke woodsholea*	12.8%	*Xylophaga* sp.	23.3%
Aricidea abranchiata	4.4%	*Capitella* sp.	11.4%	*Eulima stenostoma*	16.3%
Glycera mimica	4.2%	*Glycera mimica*	11.4%	*Prochaetoderma* sp.	4.7%
Pholoe anoculata	3.7%	*Nucula cancellata*	9.9%	Ophiuroid	4.7%
Priapulus atlantisi	3.3%	*Nucula cortica*	5.0%	*Xyloredo ingolfia*	2.3%
Nucula cancellata	3.1%	*Pholoe anoculata*	3.6%	*Dacrydium* sp.	2.3%
Polycarpa delta	2.9%	Ampharetid	3.6%	Pectenid bivalve	2.3%
Tharyx sp.	2.6%	Holothurian	2.8%	*Cocculina leptale*	2.3%
Poecilochaetus fulgoris	2.4%	*Polycarpa delta*	2.8%	*Dentalium* sp.	2.3%
				Sphaerodorid	2.3%
				Spionid	2.3%
				Epicarid isopod	2.3%
				Gnathiid isopod	2.3%

was kept at room temperature for at least 2 weeks, then frozen at −30 °C for at least 1 week, and then thawed. One box was retrieved in 1974 after it had been on the bottom for 26 months and the second box was cored *in situ*, also after 26 months, with eight 35-cm^2 tubular cores. Similar tubular cores were also used in 1972 and 1973 to obtain a total of 25 control cores from surrounding sediments at PBS 1. A third box was placed at PBS 1 in July 1975 and retrieved after 2 months. Another box of the same size, containing similarly treated Buzzards Bay mud was placed on the bottom at a depth of 10 m in Buzzards Bay in July 1971 and was sampled after 2 months with six 35-cm^2 cores. All macrofaunal animals (excluding nematodes, podocopid ostracods and copepods) retained by 0.297-mm screens were used in the analysis. Subsamples from the experimental boxes indicated no spatial patchiness as was also true in the control cores from the sea floor even though the control cores were taken at several different times.

Experimental boxes showed an order of magnitude fewer individuals and species than surrounding sediments (Table 1). The 2-month box (PBS-1, experiment 1) was dominated by larvae of the wood-boring *Xylophaga* sp. and the loricate larvae of *Priapulus atlantisi*. The abundance of *Xylophaga* sp. probably results from the proximity of wood panels placed on the same dive. All individuals of the other taxa were post-larvae or juveniles except for two adult ectoparasitic snails (*Eulima stenostoma*) which may have crawled in or been carried in by an echinoderm passing over the box. The 26-month boxes (PBS 1, experiments 2a and 2b) were dominated by juveniles of four species and adults of an as yet undescribed capitellid polychaete of the *Capitella* species complex (Table 2)[6]. The finding of a species in the *Capitella* group in the deep sea is of considerable interest, since species of this group have been recognized as indicators of disturbed conditions in shallow-water environments[7]. Only a few individuals of the *Capitella* group have previously been found in extensive collections from the deep Atlantic[8]. Hartman found large numbers of worms of similar description from collections in the vicinity of freshwater aquifers off California at depths up to 500 m (ref. 9). Polychaetes, *Glycera mimica* and *Neopodarke woodsholea*, and a bivalve, *Nucula cancellata*, were among the five numerically dominant species. An ectoparasitic gnathiid isopod was the most abundant species in the box even though it is relatively rare in surrounding sediments. Of the 141 individuals in the box sampled completely, only 18 adults were found: 16 individuals of *Capitella* sp., a single individual of the capitellid polychaete genus, *Heteromastus*, and a single adult tunicate, *Polycarpa delta*. The smaller area sampled from the box cored *in situ* showed a similar species composition.

Three of the five most abundant species in the PBS 1 26-month boxes are predators or ectoparasites and 64% of the polychaetes are in groups known to move about actively as adults; that is, most of the species settling in the boxes as larvae are relatively motile forms.

The results presented demonstrate a tremendous difference between rates of colonisation in shallow-water at a depth of 10 m and these rates in the deep sea. Comparison of two boxes sampled on the bottom (shallow-water and deep-sea) after 2 months indicates two orders of magnitude lower density in the deep-sea box. The 41 larval and post-larval individuals and two adults in the deep-sea box belong to 14 species. The shallow-water Buzzards Bay box contains thousands of individuals ranging from post-larvae to adults of at least 47 species. The difference is even more striking if the study on similar boxes placed in an intertidal mud flat is used as a basis for comparison[7]. The experiments in the intertidal zone demonstrate an even faster response with high densities of opportunistic species settling as larvae in the boxes and many growing to adults within a month. In the deep sea the densities do not rapidly increase to saturation levels. Indeed, more recent results from greater depths suggest even lower rates of colonisation. We cannot discount the possibility that densities are kept low by predation[10,11], but if this is so, the predation seems to be continuous, in that it is observed in both 2-month and 2-yr boxes, and does not result in patchy distribution within the boxes.

These results indicate that disturbance resulting in severe reduction of deep-sea fauna may be a long-lasting source of spatial heterogeneity (on the same scale as the disturbance) with the effects of disturbance apparent for at least 2 yr and probably much longer. Effects of disturbances 100 cm^2 or larger have not been observed in studies of patchiness in the deep sea, but the life and death of individual organisms can create local heterogeneity[12]. Life processes as reflected in rates of colonisation and growth are remarkably slow in the deep sea. Thus, small-scale spatial and temporal mosaics resulting from disturbances on the scale of individual organisms may have a major role in structuring highly diverse deep-sea communities.

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5-^{125}I-iododeoxyuridine as prototype for radionuclide therapy with Auger emitters

MANY of the physical and biological requirements for treating cancer with internally emitting radionuclides are met by 5-^{125}I-iododeoxyuridine (^{125}IUdR). The decay of ^{125}I is associated with a highly localised deposition of energy[1]. Cascades of very low energy and thus densely ionising Auger electrons are released. In addition, a charge transfer reaction takes place leaving the resultant tellurium nucleus with a transient mean net charge of +9 (ref. 2). These unique physical characteristics can be used for cell destruction by labelling DNA with ^{125}IUdR, a thymidine analogue. The marked radiotoxicity of ^{125}I incorporated into DNA as ^{125}IUdR has been demonstrated by lethality, mutations and DNA strand breaks in bacteria and bacteriophage[1–7], by lethality and chromosome aberrations in mammalian cells cultured *in vitro*[8–10], and by diminished survival of prelabelled tumour cells assayed *in vivo*[11]. The intranuclear location is of major importance: ^{125}I located extracellularly[9] or bound to cell membranes is much less toxic (unpublished work of R. L Warters and K. G. Hofer) These findings suggested that ^{125}IUdR might have significant anti-neoplastic activity. We tested this hypothesis in a murine ascites tumour model[12,13]. In a pilot study using therapeutic doses of ^{125}IUdR, the agent substantially augmented the median and absolute survival of treated animals. The critical factors affecting treatment of a 1-d-old ascites tumour model with ^{125}IUdR are detailed here.

The tumour used in these experiments arose spontaneously in the ovary of a C3H mouse[14] and has been maintained as an ascites tumour by serial intraperitoneal (i.p.) transplantation in female C3HeB/FeJ mice (Jackson Laboratory) Median survival of untreated animals was determined after i.p. challenge with various tumour cell inocula (Fig. 1). The non-linearity observed at low tumour cell inocula ($<10^3$ cells) suggests that host defenses may be effective in these circumstances The relatively long survival of tumour-bearing mice facilitates quantitative evaluation of tumour cell killing after treatment with ^{125}IUdR and Fig. 1 can be used to calculate a cellular survival fraction. For example, the median survival of mice given 10^7 cells is 19 d; if treatment prolongs it to 29 d, the treated animals died as though they had been given 10^3 cells. This represents a survival fraction of 10^{-4}. The calculation assumes that treatment with ^{125}IUdR does not alter the pathogenicity of tumour cells destined to survive, and the absence of a shoulder on the *in vitro* survival curve of cells exposed to ^{125}IUdR supports this assumption[8,10].

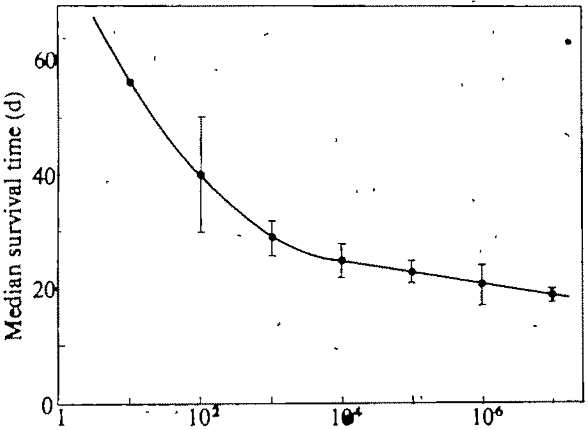

Fig. 1 Median survival ± standard deviation of untreated mice after i.p. injection of various tumour cell inocula. The median survival after injection of 10 cells represents the pooling of data from 14 experiments, in six of which fewer than half the animals died when followed for 150 d.

Parameters of the cell cycle were determined for 1-d-old ascites tumour cells by the percentage labelled mitoses method[15] (Fig. 2). From computer analysis of the data[16], the total cell cycle time was calculated to be 14.3 h, DNA synthetic time 5.8 h, G_1 6.3 h, G_2 1.7 h, and mitosis 0.5 h. Because ^{125}IUdR is incorporated into DNA only during synthesis, it is important to match fractionation to the cell cycle. As the growth fraction for 1-d-old ascites cells approaches unity, all tumour cells can theoretically be labelled by appropriate schema Optimal cell killing will be achieved by increasing the overall treatment time to cover several cell cycles and, in the absence of continuous infusion, maintaining an interval between treatments that is less than the DNA synthetic time.

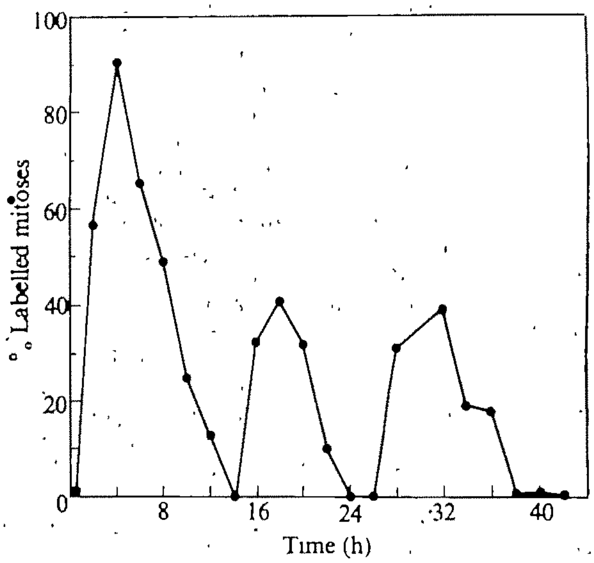

Fig. 2 Percentage labelled mitoses as a function of time after a single injection of 10 μCi ^{3}H-thymidine administered 1 d after inoculation of 10^6 tumour cells. Mice were killed at 2-h intervals; tumour cells were extracted from the peritoneal cavity by flushing, washed, fixed in methanol–acetic acid (3:1), and dropped on to slides. Autoradiography was performed with NTB-2 nuclear track emulsion (Eastman Kodak).

All experiments involved carrier-free ^{125}IUdR (New England Nuclear) administered i.p starting 24 h after inoculation of 10^6 tumour cells. The overall treatment time was 12–24 h with the interval between treatments being 4 h Because ^{125}IUdR is dehalogenated rapidly *in vivo*[17], potassium iodide was added to animal drinking water to block thyroid uptake of released radionuclide. Experiments were followed for at least 100 d No gross acute or chronic toxicity was observed in treated animals and all deaths were associated with ascites formation.

Optimal conditions for ^{125}IUdR therapy were achieved by studying the effect of sequential treatments on tumour cell survival and establishing a dose-response relationship. In Fig 3, tumour cell survival is plotted as a function of the number of treatments administered, the dose per treatment being constant The effect of using four to seven sequential treatments is to extend the overall treatment time from 12 to 24 h, an increase approximating one cell cycle time. Because ascites cells are asynchronous, increasing the number of treatments and hence the overall treatment time would be expected to reduce further the survival fraction. The incremental reduction in survival fraction decreases with each successive treatment because fewer and fewer cells remain viable to incorporate ^{125}IUdR.

Tumour cell survival as a function of the dose of ^{125}IUdR per treatment is shown in Fig 4 Seven treatments were

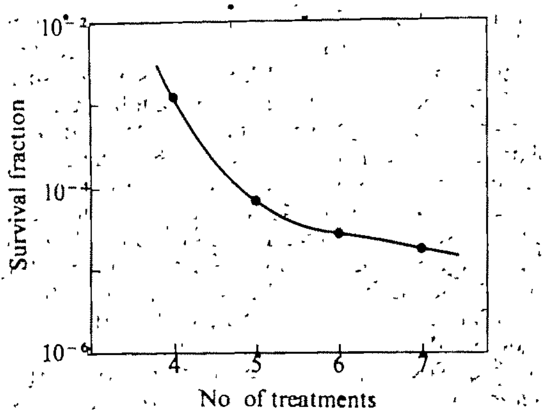

Fig. 3 Tumour cell survival as a function of sequential treatments with ^{125}IUdR (25 μCi every 4 h).

administered at 4 h intervals beginning 24 h after tumour cell inoculation. There is a steep and marked reduction in survival fraction at doses up to 15 μCi per treatment with a plateau observed at higher doses.

Both Figs 3 and 4 are complementary in that seven treatments of 25 μCi ^{125}IUdR result in a survival fraction of about 10^{-5}. Considering that the challenge dose was 10^{6} cells, these data suggest that there is a very small fraction of tumour cells that are non-cycling or have long generation times. Increasing the number of treatments and overall treatment time or the use of continuous infusion might have killed these few remaining cells but perhaps at the risk of damaging critical normal tissue cell renewal systems.

In other studies[12,13], we showed that Na^{125}I, ^{131}IUdR and Na^{131}I had no anti-neoplastic activity when administered in similar doses and using a similar regimen to that used for ^{125}IUdR. The decay of ^{131}I is by β-minus emission and is not associated with any significant yield of Auger electrons or a charge transfer reaction. No anti-neoplastic activity was observed with non-radioactive IUdR administered in amounts comparable with ^{125}IUdR. That the anti-neoplastic activity of ^{125} IUdR is primarily due to the radiotoxicity of ^{125}I incorporated into DNA is further supported by the failure to observe X-ray sensitisation in tissue culture at concentrations of IUdR that are toxic when used as ^{125}IUdR[8].

We conclude that ^{125}IUdR has marked anti-neoplastic activity and provides the basis for a new approach to the treatment of cancer. We appreciate that any early ascites tumour model is a propitious system in which to test the efficacy of cell cycle active agents. In particular, a favourable therapeutic ratio results from the direct access of drug to tumour cells in the peritoneal cavity before ^{125}IUdR enters the general circulation where dehalogenation occurs; such dehalogenation in turn protects normal cell renewal systems. Further work will be necessary to determine the efficacy of ^{125}IUdR in solid tumours where the growth fraction is considerably less than unity and drug access is less direct. Nevertheless, the exquisite radiotoxicity of ^{125}I incorporated into DNA represents a challenge to synthesise and evaluate other labelled agents with high nuclear affinity.

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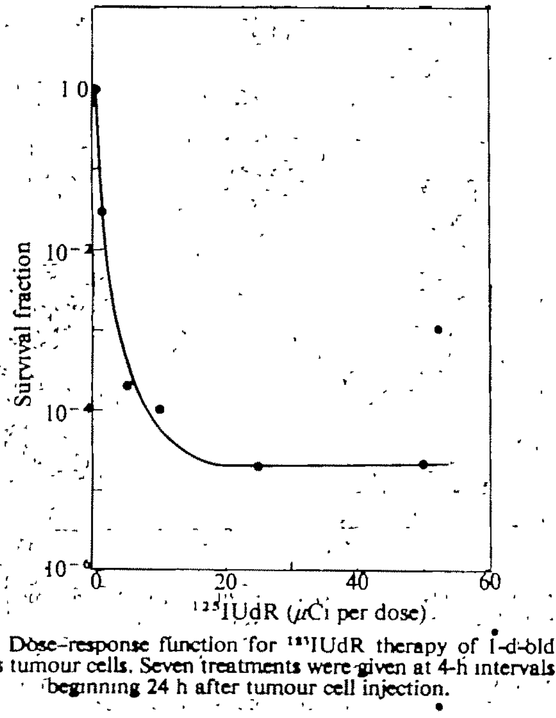

Fig. 4 Dose-response function for ^{125}IUdR therapy of 1-d-old ascites tumour cells. Seven treatments were given at 4-h intervals beginning 24 h after tumour cell injection.

First North American record of the extinct panda *Parailurus*

THE living pandas are restricted to the mountains of south-eastern Asia where both the giant panda (*Aeluropoda*) and the lesser panda (*Ailurus*) occur. They overlap in geographical range only in Szechuan, China, and there they are separated altitudinally, with the lesser panda favouring higher elevations[1]. Pleistocene records extend the range of the giant panda into Burma and eastern China, but the lesser panda has apparently not had a significantly wider range during the Quaternary[2]. Considerable controversy surrounds the phylogenetic relationships of both genera as they represent some of the most derived of terrestrial Carnivora. Recent marshalling of anatomical evidence on the giant panda has demonstrated its fundamental affinity with other members of the Ursidae[3]. The position of the lesser panda is equivocal although it is usually assigned to the Procyonidae. Procyonids are widely thought to be restricted to the New World, but recent work on various Oligocene and early Miocene forms in Europe[4] and North America[5] indicate that the procyonids had a Holarctic distribution at that time. Regardless of its broader phylogenetic affinity, the presence of medial and late Miocene relatives of the lesser panda in Pakistan and western Europe (*Sivanasua* Pilgrim, 1931 = *Schlossericyon* Crusafont, 1959)[6] and their absence in the New World makes an Old World origin for this group highly likely. Thus the discovery of a single tooth in North America almost identical to European specimens of the extinct lesser panda *Parailurus* is unexpected, and must be explained by immigration from Asia.

It has long been known that Eurasiatic mammalian immigrants had an increasingly important role in the late Cainozoic fauna of North America, and in Quaternary time such allochthonous groups came to dominate the large mammal fauna of the New World[7]. Recent study[8-10] of Pliocene (Blancan) assemblages of the north-western United States has added new taxa to the growing list of forms first appearing in the New World during that interval. The discovery of remains of the extinct panda *Parailurus* Schlosser, 1899, in the Taunton Local Fauna of the Ringold Formation of Washington adds another element to this list. It is interesting that the Ringold and correlated Glenns Ferry formations (see below) contain several mammals representative of the oriental temperate woodland fauna that appear simultaneously in Europe in the Pliocene. In the New World these immigrants include the grison *Trigonictis*[8] (close to *Enhydrictis*), the bear *Ursus* (*U. abstrusus*[8] close to *U. boeckhi*), true otters (Lutrinae, represented by *Satherium*[8] in North America) and primitive Cervidae with antlers of the 'three-point plan' of Geist[9] (*Bretzia*[10] with palmate antlers similar to Eurasiatic late Miocene *Cervavitus*). The recent discovery of the petauristine squirrel *Cryptopterus* in the Blancan of Florida[11] adds another oriental element to the Pliocene fauna of North America. In European sites yielding *Parailurus*, similar associations of mammals occur in the late Pliocene at localities of late Ruscinian (Csarnotian) age in England (Red Crag)[12], West Germany (Wölfersheim)[13] and Rumania (Barót-Köpec)[14] and those of early Villafranchian age in Italy (Arondelli[15] of the Villafranchian stratotype area), and Czechoslovakia (Hajnàcka)[16,17]. The known Pliocene faunas of Asia are of different character and seem to represent a steppe or savanna (mixed grassland and forest) biotope, that, judging from available records[18], extended northwards to the latitude of Lake Baikal. There is evidence for a Pliocene temperate forest biome that ranged at even higher latitudes across Holarctica[19], and thus a pathway is present to permit the widespread occurrence of the fauna associated with such environments.

The Taunton Local Fauna was obtained from flat-lying fluviatile deposits resembling those of the stratotype Ringold Formation at White Bluffs on the Columbia River, 24 km to the south. The higher topographic position for the Taunton site suggests that it may equate with the uppermost part of the type Ringold sequence, above the occurrence of the White Bluffs Local Fauna[10]. Fish remains are the most abundant fossils at the Taunton Site and most of these pertain to a perch (*Archoplites taylori*) and a catfish (*Ictalurus vespertinus*), both of which are common in the Glenns Ferry and type Ringold formations. Mammals are mostly known from isolated teeth. Most common are *Equus* (*Dolichohippus*) *simplicidens*, two species of the rabbit *Hypolagus*, and an antilocaprid similar in size to the living pronghorn. On the whole, the Taunton Local Fauna is similar to both the White Bluffs Local Fauna and the Hagerman Fauna. But the following differences are apparent when comparisons are made with the former: the presence of two species of *Hypolagus*, neither being the White Bluffs form; the presence of *Pliopotamys* cf. *minor* and the giant beaver *Procastoroides;* the presence of the badger *Taxidea;* the presence of *Canis lepophagus* rather than *C. davisi;* the absence of a more primitive *Equus* with short protocones; the rarity of cervids and the peccary *Platygonus*, and the presence of abundant antilocaprid remains. Some of these differences may be due to ecological bias. The stream which deposited the sediments containing the Taunton Local Fauna seems to have been a relatively minor tributary. Riparian forests that probably broadly lined the major stream at White Bluffs may also have extended as a narrow belt into the Taunton tributary with savannah or open grassland nearby. This situation would explain the large percentage of rabbits, horses and antilocaprids as well as the presence of a smaller number of presumably forest animals at Taunton. Other differences can be construed as indicating closer temporal equivalence of the Taunton Local Fauna with the younger Hagerman than with the older White Bluffs Local Fauna. Such faunas are attributed to the earliest half of the Blancan Mammal Age of North America.

Biochronologically the occurrence of a species of *Parailurus* close to *P. anglicus* in North America serves to reinforce the correlation of the early Blancan faunas of which it is a part with those of Late Ruscinian and early Villafranchian Age in Europe[20]. Radiometric calibration of these biochronological units in North America and Europe confirms this biological correlation: the Taunton Local Fauna is no younger than the Hagerman Fauna, the latter dated at 3.2–3.5 Myr (ref. 21). The early Villafranchian Etouaires Local Fauna of the Perrier Plateau, Auvergne, is just above a volcanic ash dated 3.4 Myr and is overlain by pumice dated 3.3 Myr (ref. 22). If the Vialette Local Fauna from the Auvergne is taken as Late Ruscinian in age and has a minimum date of 3.8 Myr (ref. 20) (established by the date on the overlying basalt), the Ruscinian–Villafranchian boundary would fall between 3.4 and 3.8 Myr. In round figures the known biochron of *Parailurus* would fall across this boundary probably within the interval 3.0–4.0 Myr.

The North American *Parailurus* is represented by a highly diagnostic upper right first molar (Los Angeles County Museum 10808) collected by George F. Beck of Yakima, Washington from fluviatile gravels and cross-bedded sand (284.0 m above sealevel) that in turn overlie laminated chalky clay and are overlain by late Quaternary loess, 0.8 km east of Taunton station (LACM Locality 6408, 46 41′N latitude, 119°21′W longtitude), Adams County, Washington. This tooth is longer anteroposteriorly than it

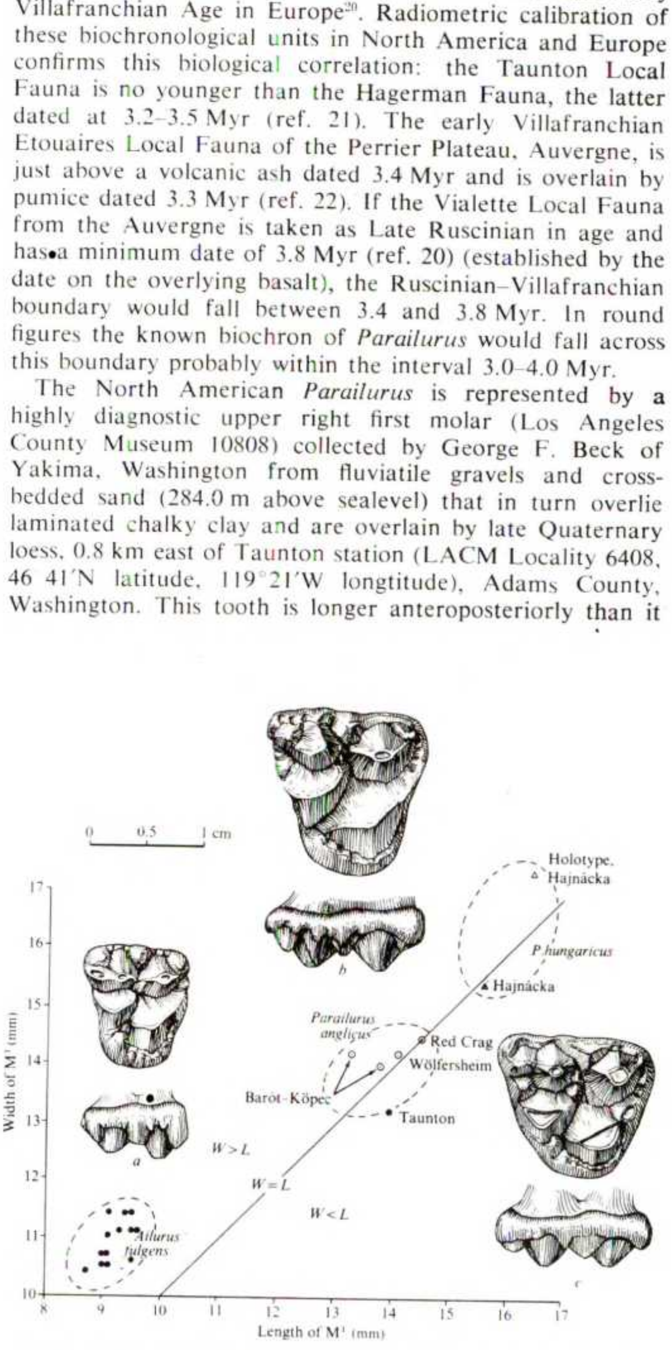

Fig. 1 Occlusal and labial views of right first upper molars of living and fossil lesser pandas, drawn to the same scale. *a*, *Ailurus fulgens* AMNH 80164, Recent, zoo; *b*, *Parailurus* cf. *anglicus*, Senckenberg Mus. Wö 21, Pliocene, W. Germany; *c*, *Parailurus* sp., LACM 10808, Pliocene, Washington. Regression plot of length × width of the first upper molar.

is wide transversely (length 14.0 mm, width 13.2 mm) in contrast to European *Parailurus* in which the teeth are more nearly equidimensional or slightly wider than long. *Ailurus fulgens*, the living lesser panda, has smaller first molars that are markedly wider than long. The complex crown pattern of the Taunton form agrees in most details with those known for *Parailurus*, as shown in Fig. 1, where LACM 10808 is compared with the specimen from Wolfersheim (Senckenberg Museum Wö 21), West Germany. The labial cingulum is weaker than in other *Parailurus* particularly that behind the mesostyle where stylar cusps form a more complex structure in *P. anglicus* and *P. hungaricus*. Likewise, the lingual cingulum is weaker in the American form giving the tooth a more triangular occlusal outline. But too few specimens of any population of *Parailurus* are known from which to judge the significance of the small differences that can be observed. The sample of *Ailurus fulgens* available (14 individuals from the American Museum of Natural History collection, three wild, the remainder zoo animals) shows a rather remarkable uniformity in size and morphology for an arctoid carnivore, suggesting that the difference in size between *P. anglicus* and *P. hungaricus* is probably significant biologically and that the morphological differences noted between the American and European forms are also significant. At present it seems most prudent to designate the American panda simply *Parailurus* sp., noting that among the described European species it compares most favourably with the late Ruscinian *P. anglicus*.

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Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors

THE colonisation of bacteria on human mucosal tissues is now recognised as an important step in the infectious process[1,2]. To colonise mucosal surfaces, the organisms must first bind to the epithelial cells of these tissues, or they are discarded by the host's physico-anatomical defence mechanism (sneezing, coughing, urine flush and so on). The attachment of bacteria to epithelial cells can be demonstrated *in vitro*[3,4] and is probably mediated by surface components of both types of cell[3]. It was found[4,5] that lipoteichoic acid on the surface of group A streptococci is responsible for the attachment of these organisms to oral mucosal cells. For Gram negative organisms, pili mediate their binding to epithelial cells[6–9]. In a study with *Proteus mirabilis* it was found[10] that the presence of pili is important for the organisms to adhere to both oral and bladder mucosal cells as well as to initiate retrograde infection in experimental animals, probably by enabling the bacteria to adhere and to colonise pelvic epithelial cells[11]. Nothing is known, however, about the putative receptor sites required for bacterial attachment on the mucosal cell membrane. We present here data indicating that attachment of *Escherichia coli* to epithelial cells is mediated by mannose (or mannose-like) receptors present on the surface of the latter.

In vitro adherence was studied by methods described previously[4]. Briefly, 0.5 ml of a suspension of epithelial cells (10^6 cells per ml in phosphate buffered saline, 0.1 M PO_4 and 0.15 M NaCl, *p*H 7.2–7.4) from the oral cavity of one of us (I.O.) was mixed with 0.5 ml of washed bacterial cells (*E. coli* strain 3092, a K_{12} derivative[12], or strain B, adjusted to 5×10^9 organisms per ml, or group A type 12 streptococci, 2×10^8 organisms per ml). The mixture was rotated end over end for 30 min at room temperature, then non-adherent bacteria were separated from the epithelial cells by differential centrifugation. The pellet of epithelial cells obtained after four washings with water was resuspended in 0.05 ml phosphate-buffered saline and placed on a slide. After drying, the preparation was stained with gentian violet and examined using light-field microscopy. Adherence was recorded as percentage of epithelial cells containing 50 bacteria or more. When the K_{12} or B strains of *E. coli* cells were mixed with a suspension of epithelial cells, the bacteria bound avidly to the cells (Fig. 1*a*) as shown previously for streptococci[4,5]. Only *E. coli* cells collected in the late stationary phase or from cultures 24–72 h old, were attached to the epithelial cells.

D-Mannose and methyl α-D-mannopyranoside (αMM) inhibited the adherence to the epithelial cells of both strains of *E. coli* but not of streptococci (Table 1). None of the other sugars tested inhibited epithelial binding of these organisms. The inhibition by αMM of adherence was dose related and was linear in the range of 2–6 mg sugar per ml. In the presence of αMM (25 mg ml^{-1}) the *E. coli* cells did not adhere; they were separated from the epithelial cells and each type of cell was washed three times to remove the αMM. On remixing the cells, epithelial attachment of the bacteria was restored, indicating that the inhibition of adherence by the sugar is reversible and probably does not involve any irreversible alterations on either type of cell.

Our observations suggest that saturation of binding sites on the bacterial surface by a sugar prevents the attachment of these organisms to the epithelial membrane receptor, which contains mannose or a mannose-like structure. This conclusion is supported by the following results. (1)

Table 1 Inhibition of adherence of *E. coli* to epithelial cells by carbohydrates, lectins and oxidation with sodium metaperiodate

Inhibitor used*	Concentration in reaction mixture (mg ml^{-1})	% Adherence†
Methyl-α-D-mannopyranoside	25	5
	5.0	25
D-mannose	25	10
	5.0	30
D-galactose	25	99
N-acetyl-D-glucosamine‡	25	85
Wheat germ agglutinin	0.5	99
Peanut agglutinin	0.5	75
Concanavalin A	0.5	28
Oxidation of epithelial cells with sodium metaperiodate§		0

*The sugars or lectins[13, 14] were incubated with suspensions of epithelial cells and bacteria for 30 min at room temperature, then treated as described in text.

†This was calculated as percentage adherence in the presence of sugars or lectins compared with control (without inhibitor) which was taken as 100% adherence.

‡The percentage adherence in the presence of other sugars (25 mg ml^{-1}) was in all cases >90%. The sugars tested were: D-rhamnose, L-fucose, maltose, D-glucose, methyl-α-D-glucopyranoside, and methyl-α-D-galactopyranoside.

§An epithelial cell suspension (0.5 ml, 10^6 ml^{-1}) was incubated with 10 mM of sodium metaperiodate for 10 min at room temperature. The cells were then washed twice with phosphate-buffered saline, resuspended in 0.5 ml of the same buffer, and mixed with *E. coli* suspension as described in the text.

Epithelial cells treated with sodium metaperiodate, a reagent known to cleave the C–C bond between vicinal hydroxyl groups of sugars, completely inhibited the binding of *E. coli* (Table 1) but did not affect binding of streptococci. (2) Concanavalin A, which is known to bind to D-mannose and D-gluscose residues[13], inhibited attachment to cell surfaces of *E. coli* but not of streptococci. The other lectins tested (wheat germ agglutinin—specific for *N*-acetyl-D-glucosamine (ref. 13), and peanut agglutinin—specific for D-galactose (ref. 14)) did not inhibit the binding of *E. coli* or of streptococci. (3) Both soluble yeast mannan and intact yeast cells, which are known to contain mannans on their cell surface[15], agglutinated *E. coli* cells (Fig. 1*b* and *c*). Incubation of a suspension of *E. coli* with mannan (7 μg ml^{-1}) or 10^5 yeast cells per ml, gave visible agglutination of the bacteria after 30 min, characterised by a diffuse carpet of cells on the bottom of the tube. Agglutination of *E. coli* by both mannan and yeast cells (*Saccharomyces cerevisiae*) could be completely blocked by D-mannose or by αMM at a concentration of 25 mg ml^{-1}, but not by any other sugar tested at the same concentration.

Addition of D-mannose or αMM (25 mg ml^{-1}) to epithelial cells to which *E. coli* was preattached caused rapid (within 2–5 min) displacement of the organisms from the epithelial cells. After washing the epithelial cells four times with a solution containing αMM (6 or 12 mg ml^{-1}), 55 and 85%, respectively, of the preattached bacteria were removed from the cells. Likewise, mannan (2 mg ml^{-1}) removed bacteria attached to the epithelial cells (Fig. 1*b*), but D-glucose, glycogen or D-galactose did not displace the bacteria.

Our results suggest that the binding of *E. coli* to epithelial cells is mediated by a mannose-specific, lectin-like substance present on the surface of *E. coli* which binds to a mannose-like receptor site on the mucosal cell. Indeed, extraction of *E. coli* K_{12} cells (1 g wet weight) with phosphate-buffered saline, adjusted to *p*H 8.5 (1 ml) at room temperature, gave a solution which contained a lectin-like substance specific for mannose, as shown by the following findings: (1) The extract agglutinated yeast cells at a dilution of 1 : 20; (2) this agglutination was inhibited by D-mannose or αMM (3 mg ml^{-1}) but not by any of the other sugars listed in Table 1 (25 mg ml^{-1}); (3) the extract, at 1 : 80 dilution, inhibited attachment of *E. coli* to epithelial cells; (4) the latter inhibitory activity was abolished by adsorption with yeast cells; (5) the agglutinating activity of the extract was stable to 59 °C (30 min), but was destroyed at 70 °C (30 min), or by treatment with trypsin (0.3 mg ml^{-1}, 2 h, 37 °C). Further work on the purification and characterisation of the *E. coli* lectin-like substance is in progress.

Production of mannose-specific lectins by members of Gram negative organisms, including strains of *E. coli*, has been reported[16–19]. In most of these studies the target host cell was the erythrocyte. Duguid *et al.*[16] reported that mannose inhibited binding of *E. coli* to intestinal epithelial cells, but this was not investigated in any detail and was overlooked in subsequent studies[2]. In agreement with the epithelial binding reported here, the bacterial haemagglutination was very weak in the logarithmic phase of growth and became strong only at 24–48 h growth[19].

Our report provides evidence suggesting that the mannose-specific bacterial haemagglutinins[16–19] enable the organisms to attach and colonise human mucosal tissues. Furthermore, mannose and its derivatives, which act as receptors for *E. coli* binding, can compete with the epithelial membrane-bound mannose to dissociate the organisms from the cell surfaces. Studies with concanavalin A suggest that mannose is widely distributed on the membrane of mammalian cells[13]. It would seem therefore that the normal distribution of Gram negative organisms on different mammalian mucosal tissues is not due to the presence or lack of the mannose receptor on epithelial cells. This is supported by the findings that, although the ileum rather than the jejunum of the gut is normally colonised by *E. coli*, the epithelial cells from both regions

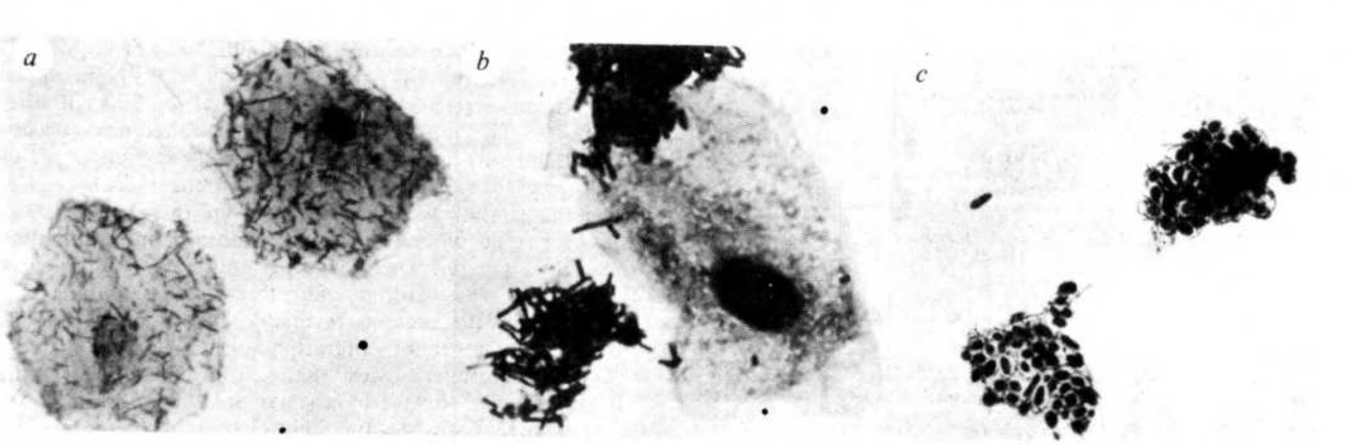

Fig. 1 Binding of *E. coli* to: *a*, human epithelial cells (×400); *b*, yeast mannan (Sigma) after removal from epithelial cells (×1,000); and *c*, yeast cells (*Saccharomyces cerevisiae*).

bound *E. coli* equally well[19]. The mechanism of attachment of streptococci to epithelial cells is different from that of *E. coli*, for none of the sugars tested inhibited the lipoteichoic acid-mediated streptococcal attachment[4,5]. In this regard it has been suggested that certain human tissues, such as sections of heart valves, lack receptors for *E. coli* but not for streptococci[21].

In conclusion, our finding that mannose, a sugar found on most mammalian cell surfaces, acts as receptor for binding of *E. coli*, which is one of the most common commensals of man, may provide an approach to the elucidation of the mechanism of bacterial adherence and subsequent colonisation on mucosal tissues.

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Suppression by heparin of smooth muscle cell proliferation in injured arteries

INTIMAL smooth muscle cell (SMC) proliferation dominates the early phase of healing after arterial endothelial injury[1-6]; what growth factors are responsible for this mitotic activity of an otherwise quiescent cell is not known. SMC growth *in vitro* is enhanced by platelet factors, insulin and lipoproteins[7-11] and, according to some reports, can be diminished *in vivo* with various antiplatelet agents. Harker *et al.*[8] have reported decreased myointimal thickening in homocystinaemic baboons treated with dipyridamole, and Friedman *et al.*[11] and Moore *et al.*[12] have reported suppression of SMC proliferation in the injured arteries of rabbits injected with anti-platelet serum. Intimal thickening was not diminished, however, in the injured carotid arteries of rats given various anti-platelet drugs[13]. Because the clotting system is inextricably linked to arterial injury and because thrombin is used to generate the platelet growth factor[9] and may itself be a mitogen[14-16], we speculated that heparin might inhibit myointimal thickening. We report here the use of heparin to suppress intimal SMC hyperplasia in a rat model of arterial endothelial injury and suggest possible mechanisms for the heparin effect.

Endothelial injury was achieved by infusing air into isolated segments of right carotid arteries of 45 young male Sprague–Dawley rats (250–300 g, Charles River Breeding Laboratories) as described earlier[1,2]. This procedure dessicated the endothelium. After blood flow was re-established and haemostasis obtained, the wound was irrigated with Betadine solution. Using a sterile technique, a silastic catheter was inserted into the left jugular vein and connected to an infusion system as described by Steiger *et al.*[18]. Initially all rats were infused with lactated Ringer's solution (Abbott) at 0.91 ml h^{-1} using a syringe infusion pump. After 24 h, experimental rats were switched to an infusion of heparin (from pig intestinal mucosa, Elkins-Sinn). Control rats were infused continuously with Ringer's solution. Animals were killed at 5, 10 and 14 d by perfusion fixation[1,2] after blood had been collected for clotting times[17] and platelet counts. Carotid arteries were further fixed and processed as described earlier[1,2] for light, scanning (SEM) and transmission electron microscopy (TEM).

Clotting times were prolonged in a predictable fashion; as noted before[18], a linear relationship existed between the logarithm of the clotting time and the heparin dose. Heparin doses of 50 or 100 U kg^{-1} h^{-1} given intravenously yielded clotting times of 12.5 and 36 min (control 4.4 min). Platelet counts were not affected by heparin administration (control. 1.28 ± 11 × 10^6 (± s.e.); 50 U kg^{-1} h^{-1}: 1.38 ± 0.04 × 10^6; 100 U kg^{-1} h^{-1}. 1.38 ± 0.11 × 10^6).

In earlier studies on uncatheterised control animals[1,2], we showed that after carotid injury the dessicated endothelium rapidly sloughed and was supplanted by a carpet of platelets. Endothelial regeneration proceeded from normal endothelium at the ends of the injured segment and was complete by 14 d; between 5 and 14 d smooth muscle cells migrated through the internal elastic lamina and proliferated in the intima. The intimal thickness was maximal at 14 d.

The right carotid arteries of catheterised control animals at 5 d demonstrated partial endothelial regeneration and a dense monolayer of platelets in the residual central denuded portion of the injured segment. Unlike uncatheterised controls, at 10 and 14 d, endothelial regeneration in catheterised control carotids was still incomplete in the central zone; in place of endothelium and platelets, modified smooth muscle cells lined the luminal surface. A possible explanation for this failure of endothelial regeneration is that in stressed animals or after long arterial injuries[4,11], rapidly growing SMC can temporarily form a luminal surface and impede endothelial regeneration. At 10 d, all injured carotids showed marked intimal thickening due to SMC proliferation; at 14 d, the intimal thickening was even more pronounced. The injured right carotids of heparin-treated rats exhibited the same features as catheterised controls at 5 d with partial endothelial regeneration at the ends and a dense carpet of platelets in the middle of the injured segment; the platelets in heparin-treated rats were morphologically (SEM and TEM) similar to controls. At 10 d, a small central residual zone of denudation covered by platelets remained and at 14 d endothelial regeneration was complete as in uncatheterised controls. At 10 d there was only slight evidence of intimal thickening which nevertheless was not significantly different from controls. But, at 14 d, because of increased intimal thickening in control carotids, the maximum ratio of intima to media (I/M) in carotids from heparin-treated rats was diminished significantly (Fig. 1 and Table 1). The

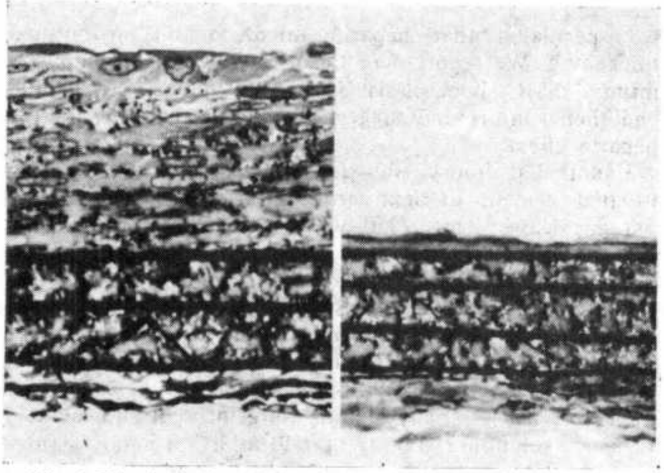

Fig. 1 Histological sections of injured right carotids in heparin-treated (right) and control (left) animals at 14 d. The maximum I/M ratio is 1.8 (control) and 0.2 (heparin). The minimal intimal thickening shown here in the right carotid of the heparin-treated rat is representative of findings in animals with a pronounced response to heparin; as can be seen in Table 1 there was considerable variation in intimal thickening under heparin treatment. TEM of these samples demonstrated only smooth muscle cells in the intima of the control carotid and a single layer of smooth muscle cells covered by endothelium in the intima of the carotid from the heparin-treated rat.

maximum I/M ratios for high and low dose heparin were similar and both were significantly different from control values. At most, one to three SMC layers were seen at this stage in the intima under the endothelium. The normal rate of endothelial regeneration in heparin-treated animals may have been related to the absence of luminal SMC.

These results indicate that heparin in doses sufficient to prolong clotting markedly suppressed intimal SMC proliferation after arterial endothelial injury even though endothelial regeneration (mitosis and migration) progressed unimpaired. Heparin did not seem to inhibit platelet-subendothelium interaction in the injured carotid.

This suppression of SMC proliferation may be the result of one or more effects of heparin because heparin is known to have many biological activities[19-21]. Heparin may act directly on the SMC[19,21]. Alternatively, because of its charge, heparin may bind and inactivate platelet growth factors generated by platelet activity on the denuded arterial wall; this is particularly plausible in view of Ross's *in vitro* results demonstrating that the SMC platelet mitogen is a basic protein[22]. Heparin may achieve several effects indirectly through its action on the clotting system. Thrombin and the other serine proteases of the clotting system (XII, XI, IX, X and plasmin) are undoubtedly activated at the surface of the denuded segment and may accumulate in the arterial wall. One or more of these clotting factors may be mitogenic for SMC[14,15]; thrombin, in particular, may generate mitogens from the adherent platelets as suggested by *in vitro* work[9,22]. Antithrombin III normally regulates the level of proteolytic activity by slowly inactivating the clotting proteases; this process is almost instantaneous in the presence of heparin[23]. Thus, heparin through antithrombin III may affect the degradation of platelets and generation of platelet SMC growth factors by thrombin[24], and the direct activity of the clotting proteases on SMC. Arterial SMC proliferation may be just one example of the general phenomenon of wound healing suppressible by heparin; previous studies of wounds in skin, bowel, artery and bone indicate that heparin can prevent scarring if given in doses large enough to produce a continuous and significant state of anticoagulation[25-28].

Table 1 Myointimal thickening at 14 d

	I/M
Control	1.9 ± 0.5 (5)
Heparin (50 U kg^{-1} h^{-1})	0.4 ± 0.2, $P = 0.02$ (5)
(100 U kg^{-1} h^{-1})	0.6 ± 0.1, $P = 0.005$ (10)
Total	0.5 ± 0.1, $P = 0.0007$ (15)

I/M ± s.e. was measured in right carotid arteries, 14 d after injury, which were cross sectioned at 500-μm stages along their entire length[2,13]. Because the thickened intima at 14 d is almost entirely composed of smooth muscle cells[1-6] (Fig. 1), the maximum I/M ratio reflects the amount of SMC proliferation and is measured by using an ocular graticule by taking the ratio of the maximum intimal thickness (distance from the luminal surface to the internal elastic lamina) to the adjacent media thickness (distance from the internal elastic lamina to the most external elastic lamina). Numbers of rats in each group are shown in parentheses.

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In vitro culture and differentiation of normal mouse blastocysts

WHEN preimplantation mouse embryos are transplanted to extrauterine sites, up to 20% develop almost normally to the egg cylinder stage (equivalent to about 6–7 d *in utero*). They then become disorganised and give rise to tumours, which are usually mature teratomas containing a wide range of differentiated tissues, but less frequently are progressively growing teratocarcinomas containing nests of undifferentiated pluripotent embryonal cells[1,2]. There are many reasons why it would be interesting to reproduce such normal and abnormal development efficiently *in vitro*. Although a number of techniques have been used to culture preimplantation embryos, none of them has proved entirely satisfactory[3-5]; in the best conditions only 5–20% of 3.5–4-d blastocysts develop *in vitro* to form differentiated, foetal tissues. Most only give rise to extraembryonic endoderm and its associated mesoderm; these cell types proliferate extensively *in vitro*, and consequently most of the continuous lines derived from blastocyst cultures have probably originated from extraembryonic tissues[6]. In this paper we describe a simple and reproducible method for culturing mouse blastocysts *in vitro* so that around 70% give rise to colonies which continue to grow for up to 4 weeks and produce a variety of differentiated tissues, including skin, nerve, beating muscle, cartilage and fibroblasts. The method relies on the technique of immunosurgery[7] for killing and removing first the trophectoderm and then the hypoblast (primary endoderm) layers of the embryo, to yield clumps of isolated epiblast (embryonic ectoderm) (Fig. 1).

What subsequently happens to the epiblast cells depends entirely on the exact conditions in which they are grown. When they are incubated in bacteriological dishes so that they cannot adhere to the substratum they degenerate within 24 h. When incubated in tissue culture dishes, however, they rapidly adhere and spread out to form a sheet of cells surrounding a central clump (Fig. 2*a*); at 48 h some cell division is still occurring, but each colony finally degenerates by about day 4. The viability of these cultures cannot be improved by coating the dishes with gelatin.

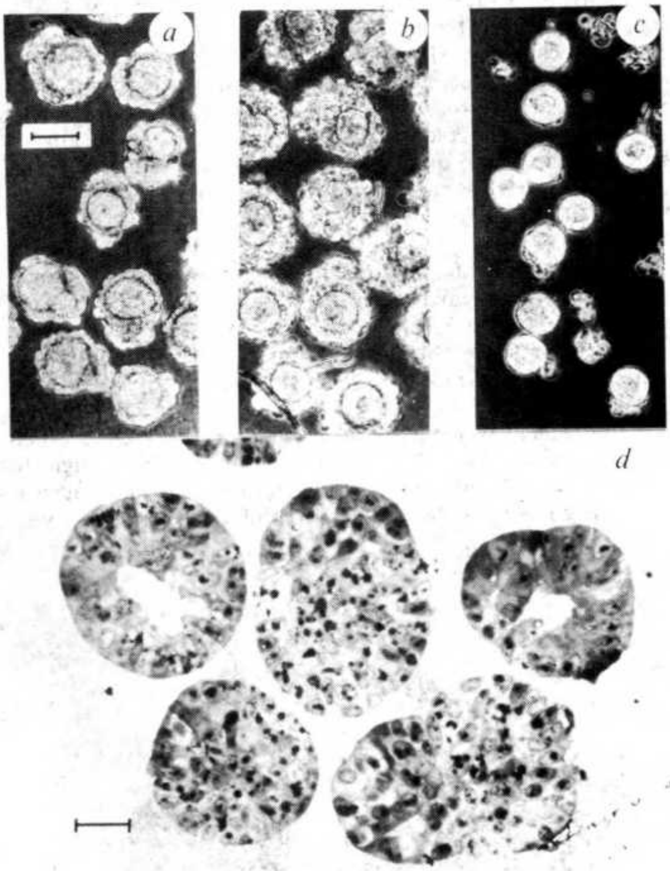

Fig. 1 Cultured embryos before and after removing the hypoblast layer by immunosurgery. Blastocysts are flushed from the uteri of C3H mice on day 4 of pregnancy and, after removal of the zonae pellucidae with Pronase[8], are incubated overnight at 37 C in a humidified atmosphere of 95% air/5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) with either 10% foetal calf serum or 20% human cord serum (HCS, heat inactivated for 30 min at 56 C). Next day, the embryos are transferred to DMEM containing rabbit anti-C57BL mouse spleen antiserum[7] diluted 1:50. After 30 min at 37 C the embryos are washed twice in DMEM with 0.5% bovine serum albumin (BSA, Sigma fraction V) and transferred to a solution of guinea pig complement (Wellcome, 1:10 dilution) and incubated for 30 min at 37 C, by which time all the outer trophectoderm cells have lysed. The embryos are washed once in DMEM plus 0.5% BSA and incubated in DMEM with 20% HCS. After 48 h all the inner cell masses have developed an outer layer of hypoblast cells and in some cases the inner epiblast cells have formed a hollow sphere (*a*). If the immunosurgery procedure is repeated the hypoblast cells are lysed (*b*) and can be removed by pipetting the embryos up and down in a finely drawn Pasteur pipeett (*c*). Most of the isolated epiblast cell masses are solid, a few are hollow and a very few are asymmetric, with a cluster of larger, more densely staining cells to one side (*d*). Of 27 epiblast masses serially sectioned at 1 μm, all had an almost continuous outer layer of basement membrane material and the few hypoblast cells still attached were clearly dead. *a*, *b* and *c*, Phase-contrast microscopy (bar: 100 μm); *d*, isolated epiblast cells, fixed in 2.5% glutaraldehyde post-fixed in OsO_4 and embedded in Araldite. Sections were cut at 1 μm and stained with Toluidine blue (bar: 25 μm).

When the stripped embryos are incubated in the presence of a monolayer of mouse feeder fibroblasts about 70%

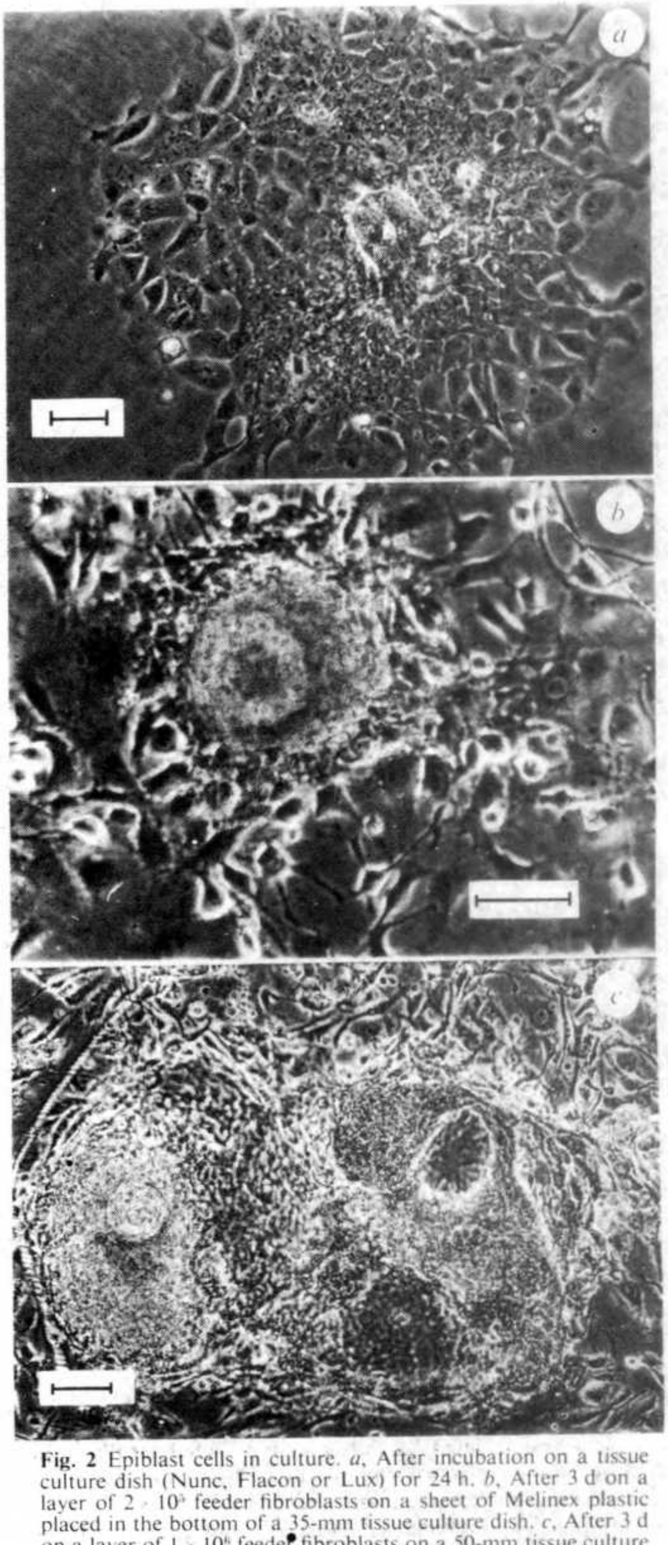

Fig. 2 Epiblast cells in culture. *a*, After incubation on a tissue culture dish (Nunc, Flacon or Lux) for 24 h. *b*, After 3 d on a layer of 2×10^5 feeder fibroblasts on a sheet of Melinex plastic placed in the bottom of a 35-mm tissue culture dish. *c*, After 3 d on a layer of 1×10^6 feeder fibroblasts on a 50-mm tissue culture dish. All cultures were incubated in DMEM containing 20% HCS. The mouse fibroblasts were treated before plating out with Mitomycin C (10 μg ml^{-1}) for 3.5 h so that they could not divide. In a typical experiment, 20–30 epiblast cell masses were added per dish and the medium changed every 2 d. Phase-contrast microscopy (bar: 100 μm).

of them survive for up to 4 weeks and form colonies containing a variety of differentiated tissues. Even in the presence of the feeder layer, however, the epiblast cells are sensitive to the underlying substrate; to facilitate electron microscope studies, the stripped embryos were sometimes grown on feeder layers on Melinex plastic (Boyden Data Papers, London, UK) and on this substratum they form rounded and compact colonies (Fig. 2*b*). When the epiblast cells are placed on feeder layers on standard tissue culture plastic, they form colonies which are much more spread out, grow more rapidly in a disorganised way and form a variety of differentiated tissues (Fig 2*c*).

Nineteen embryos which had been incubated on Melinex for 3 or 4 d were serially sectioned and examined in the light and electron microscope. The colonies fell into three main categories (Fig. 3). The simplest consists of a flattened vesicle of cuboidal or columnar cells, often embedded in the feeder layer (Fig. 3*a*). The second category contains a similar hollow vesicle of cells but, in some planes of sections, this is seen to be disorganised and surrounded by clusters of other cells (Fig. 3*b*). The third category contains in addition, a second vesicle lined by flatter cells which laterally interdigitate and have more endoplasmic reticulum and microvilli. Both vesicles are surrounded by a thin basement membrane. The cuboidal cell vesicle often contains masses of cellular debris, sugesting that it is initially formed by cell death. The flatter cell vesicle contains floccular material, suggesting that it is secretory. The cells surrounding these vesicles have few intracellular organelles and are either in tightly packed clumps, or are stellate and touch only at limited areas, often through contact zones; the stellate cells closely resemble mesodermal cells of normal 8-day embryos[14]. At the surface of the colonies some of the cells are occasionally joined laterally by tight junctions and desmosomes to form an outer layer, but this does not resemble morphologically the primary endoderm layer formed when isolated inner cell masses are incubated *in vitro* (Fig. 1*a*).

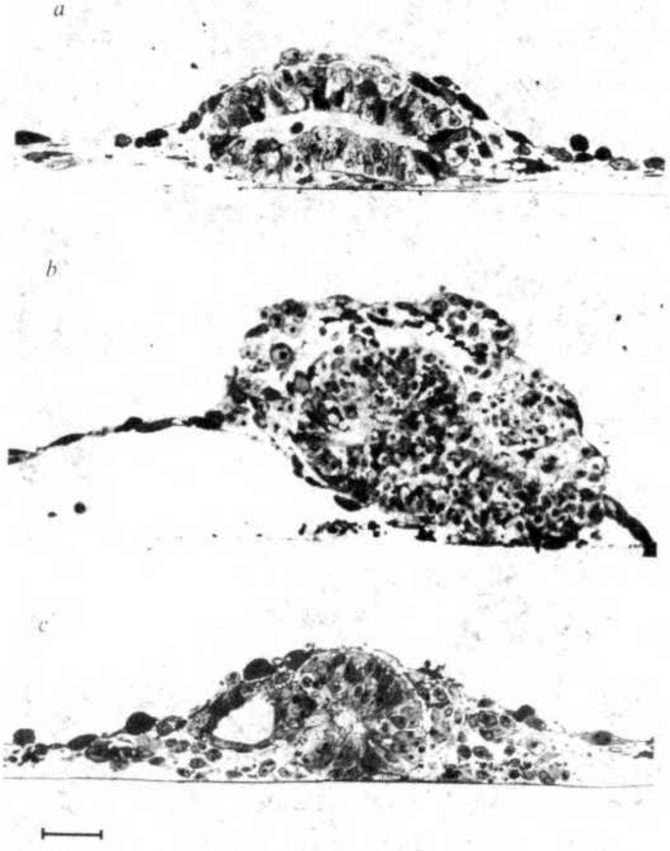

Fig. 3 Sections of epiblast cells grown on feeder fibroblasts on Melinex plastic, showing the three commonest forms of internal organisation. Epiblast cells were cultured for 3 d (*b*) or 4 d (*a* and *c*) and then fixed, sectioned and stained as described in Fig. 1*d*. In *a* and *c* the feeder fibroblasts form an almost continuous layer over the embryo; in *b* the embryo cells are on top of the feeder layer, which has partially detached from the Melinex (bar: 50 μm).

Colonies growing on feeder layers on tissue culture dishes are more spread out and, during the first few days of culture, can be seen to contain closely packed cells which resemble undifferentiated teratocarcinoma cells. Patches of fibroblast-like cells appear in most colonies by 4–5 d, together with convoluted ridges and vesicles of epithelial cells. Beating muscle and neurones are seen in about 20% of the colonies after a week in culture. Later many colonies contain clumps of large, rounded cells and also flattened cells with very large nuclei and granular cytoplasm. By 3 weeks, extensive sheets of fibroblast and epithelial cells may be seen. Seven colonies were examined histologically at around this time; all contained simple and squamous epithelium overlying mesenchymal tissue. Keratin pearls were found in two (Fig. 4*a*) and muscle and cartilage in one (Fig. 4*b*).

The experiments described here establish that isolated epiblast cells are able to differentiate *in vitro* into a variety of tissues. It seems, however, that this only occurs if the cells retain some three dimensional association following their isolation because if they are allowed to spread out into a monolayer they do not differentiate (Fig. 2*a*). The function of the feeder layer may be to provide growth factors which allow epiblast cell division to continue fast enough to counteract cell migration. If the feeder cells and embryos are plated out into separate halves of the same dish, most

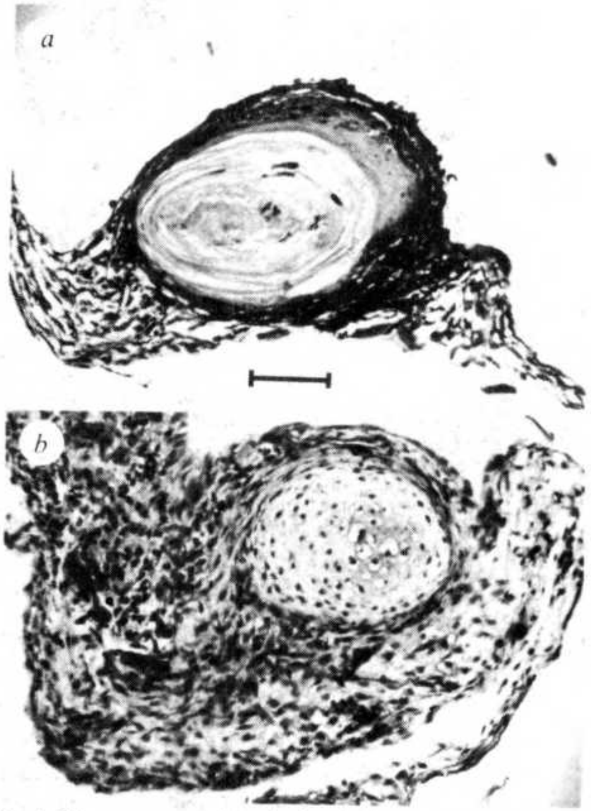

Fig. 4 Tissues present in colonies derived from epiblast cells incubated as described in Fig. 2*c*. Colonies were scraped off the dish, fixed in formal saline and embedded in wax. Sections were cut at 4 μm and stained with haematoxylin and eosin. *a*, Section showing a pearl of keratinised squamous epithelium in a colony which had been growing for 22 d. *b*, Section showing cartilage and muscle in a colony which had been growing for 17 d (bar: 50 μm).

of the epiblast cell masses still spread out and die, but a few (2/20) retain central clumps of cells and give rise to nerve, muscle, fibroblasts and skin.

The behaviour of isolated epiblast cells in culture provides several pieces of information about the way in which normal embryogenesis may be controlled. For example, the fact that mesoderm forms in the complete absence of a hypoblast layer shows that signals from the endoderm cells are not required for mesoderm differentiation. Similarly, because the epiblast cells do not reform an outer layer of primary endoderm, this suggests that either they are irreversibly committed to forming tissues other than endoderm; or they are not receiving the correct stimulus for this step under our culture conditions.

Several laboratories have studied the fate of embryonic ectoderm dissected manually from 8 d rat[11] or 6–7-d mouse[12,13] embryos and transplanted into extrauterine sites. In these sites the cells differentiate into teratomas and teratocarcinomas containing ectodermal and mesodermal tissues, as well as definitive endoderm tissues such as gut. This suggests that the endoderm of the mammalian foetus, like that of the chick[14], is being derived from cells of the epiblast and not from the hypoblast. So far we have not positively identified tissues of the definitive endoderm in our cultures, but it is possible that the formation of two vesicles in some colonies (Fig 3c) represents an attempt by the cells to make definitive endoderm

We are now using the method to isolate lines of early embryonic cells, including undifferentiated pluripotent cells equivalent to the embryonal cells of teratocarcinomas. The method may also provide a way of deriving cell lines from parthenogenetic embryos and from embryos of mice with mutations at the *T* locus.

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Parathormone-stimulated resorption of devitalised bone by cultured osteoclast-type bone cells

We recently reported the isolation from mouse calvaria of two different bone cell populations that express unique changes in metabolism when exposed in culture to parathormone and calcitonin[1–3]. In one cell type, termed *CT*, parathormone increased the synthesis of hyaluronate and the activity of acid phosphatase. In the second cell type, termed *PT*, parathormone inhibited citrate decarboxylation and decreased the activities of prolyl hydroxylase and alkaline phosphatase. Calcitonin blocked the effects of parathormone in the *CT* cells but not in the *PT* cells. Since the changes elicited in the separate populations by parathormone closely resembled those exhibited by calvaria maintained in organ culture[4], it seemed probable that the isolated cells retained unique properties in culture that are characteristic of their function in the intact tissue. On the basis of these results the *CT* cells were provisionally identified as osteoclasts and the *PT* cells as osteoblasts[3]. The major physiological result of the action of parathormone on bone *in vivo* or in culture is resorption of the calcified extracellular matrix, so we evaluated this function in the isolated bone cells. Should either the *CT* or *PT* cells promote resorption *in vitro*, this would further demonstrate that they retain differentiated function in culture and would aid in establishing their identity. We describe here a procedure which allowed us to test for resorptive properties of our isolated bone cell populations. We found that parathormone markedly stimulated the capacity of *CT*, but not *PT*, cells to resorb a calcified bone matrix substrate—a property in keeping with the identification of the *CT* cells as osteoclasts.

CT bone cells (populations 1 and 2) and *PT* bone cells (populations 4 and 5) were isolated from calvaria of 2–3-d-old mice and were maintained in primary culture for 7 d as described before[3]. They were then resuspended at 2×10^6 cells per ml in BGJ$_b$ medium[5] (Gibco), containing 5% foetal calf serum. Devitalised calcified matrix substrates were prepared from the calvaria of 3-d-old mice. After removal of extraneous tissue the calvaria were frozen at -20 °C for 3–5 d. Before use they were thawed at room temperature, then refrozen and thawed three times. In some experiments the starting calvaria were prelabelled *in vivo* with ^{3}H-proline and ^{3}H-hydroxyproline by obtaining them from pups of mothers that were injected with 250 µCi of [2,3-^{3}H] proline 1–2 d before delivery. These substrates were shown to be devitalised by their inability to resorb in culture conditions or to incorporate exogenous ^{3}H-thymidine and ^{3}H-leucine. The calvarial substrates were placed in individual wells of multi-well culture dishes (Linbro) and maintained in 5% CO$_2$ in air at 37 °C for 1 h. A 0.05-ml aliquot of the bone cell suspension (10^5 cells) was deposited in the concavity of each calvarium. Medium without cells served as a control. The cells and matrix were then incubated for 2 h (5% CO$_2$ in air; 37 °C) to facilitate cell adherence, after which they were covered with 1.0 ml of fresh BGJ$_b$ medium alone or containing parathormone 200 ng ml^{-1} (2×10^{-8} M) or calcitonin 110 ng ml^{-1} (3×10^{-8} M), or both. In some experiments 2.5×10^6 d.p.m. of ^{45}CaCl$_2$ at an initial specific radioactivity of 1.6×10^6 d.p.m. µmol^{-1} were included in this addition. The initial concentrations of calcium (Ca) and inorganic phosphate (Pi) in the incubation medium were 1.6 and 1.0 mM, respectively, and the *p*H was 7.4.

Culture was continued for 72 h. Histological examinations (our unpublished data) showed that the cells remained viable and formed a compact layer closely apposed to the surface of the bone matrix. Occasionally large multinucleated cells were seen in both populations, but more often in the *CT* layers. The medium was separated from the bone and analysed for Ca and Pi (ref. 6), ^{45}Ca and *p*H (at 37 °C, gas phase 5% CO$_2$ in air). To determine ^{3}H-proline and ^{3}H-hydroxyproline, samples of the media were hydrolysed for 24 h with 6 N HCl and samples of the hydrolysate were dansylated and chromatographed on thin-layer polyamide sheets[7] with carrier dansylproline and dansyl-hydroxyproline. The proline and hydroxyproline spots were cut out, eluted with 0.5 ml of 50% pyridine and radioactivity was assayed. The cells affixed to the calvarial matrix were tested after 72 h of culture for their capacity to synthesise ^{3}H-hyaluronate from [6-^{3}H]glucosamine and to decarboxylate [6-^{14}C]citrate, as described previously[3]. All assays of radioactivity were performed by liquid scintillation spectrometry[8].

Figure 1 summarises the results of growing bone cells on the bone matrix substrate for 72 h. With either the *CT* or *PT* cell populations in contact with the matrix there was release of Ca and Pi into the medium. In cultures of *CT* cells, however, there was substantially more ^{3}H-hydroxyproline release from

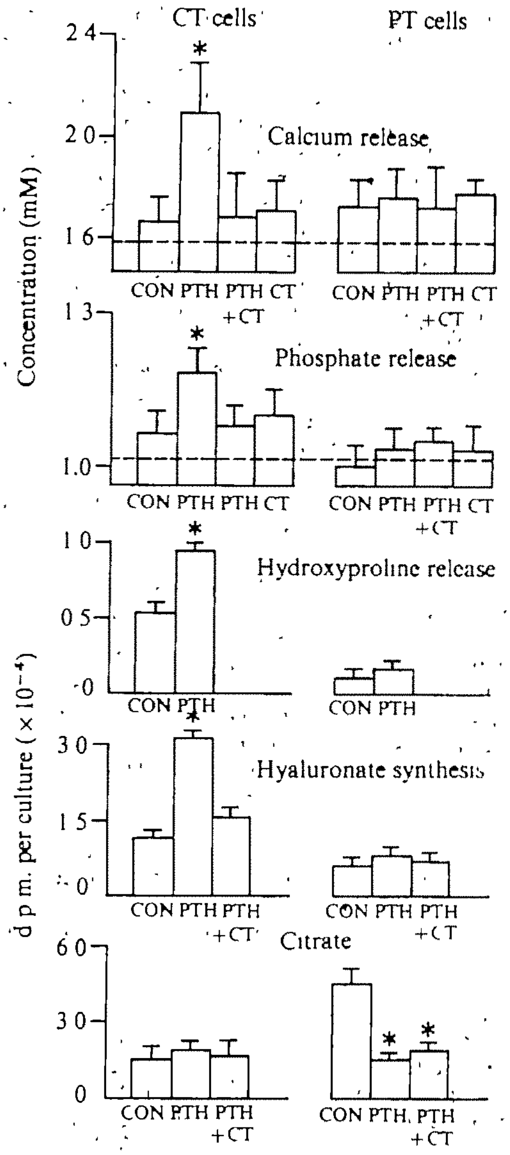

Fig. 1 Differential effects of parathormone and calcitonin on bone resorption by osteoclast-type (*CT*) and osteoblast-type (*PT*) isolated bone cell populations *CT* and *PT* cells were isolated and subcultured (10^6 cells per culture) on devitalised bone matrix for 72 h as described in the text. CON, no additions to the medium; PTH, parathormone 2×10^{-8} M; CT, calcitonin 3×10^{-8} M, PTH + CT, both hormones. Values are shown as means ± s e m of three to four cultures Baseline values shown for calcium, phosphate and hydroxyproline are those obtained when devitalised calvaria were cultured for 72 h without cells. Dashed lines denote the initial concentrations of calcium and phosphate in the BGJ_b culture medium. * = significant difference from CON, $P < 0.05$ (Student's *t* test)

prelabelled bone matrix. This indicates that in *CT* cultures resorption of collagenous matrix accompanied the dissolution of bone mineral to a greater extent than in *PT* cultures. This difference in resorption could not be correlated with differences in acidification of the medium by the cells (final *p*H in all cultures was approximately 7 2) or with differences in the uptake of ^{45}Ca by the matrix (final specific radioactivity in all cultures was approximately 0.9 d.p.m. μmol^{-1}). In cultures of matrix incubated without cells, there was a net transfer of Ca and Pi from the medium to the matrix. The *p*H did not change and no ^{3}H-hydroxyproline was released to the medium.

Parathormone markedly stimulated the resorption of Ca and Pi by the *CT* cells and calcitonin blocked this action (Fig. 1) The hormone also stimulated the release of hydroxyproline. These changes in resorption were not accompanied by any alteration in *p*H of the medium or uptake of ^{45}Ca. In contrast, parathormone or calcitonin alone or in combination exerted no significant effect on resorption of Ca and Pi by the *PT* cells. Figure 1 also shows that parathormone exerted its previously observed characteristic effects on the *CT* and *PT* cell populations[4]. That is, it stimulated hyaluronate synthesis in the *CT* cells but not *PT* cells and calcitonin blocked this action Parathormone inhibited citrate decarboxylation in the *PT* cells but not in the *CT* cells and calcitonin did not block this effect.

To establish that the action of parathormone was specific, we tested unrelated peptides and H_2O_2-oxidised (that is, biologically inactive) parathormone[9]. In three experiments, ACTH (1 U ml^{-1}), human somatotropic hormone (1 μg ml^{-1}), angiotensin II (1 μg ml^{-1}), and oxidised parathormone (1 μg ml^{-1}) were added to *CT* cells in culture with devitalised matrix as described above. Native parathormone was the control. In each experiment these peptides failed to elicit removal of calcium from the matrix by the *CT* cells.

These observations reinforce our earlier conclusion[4] that *CT* cells exhibit the characteristics of osteoclasts The findings that the resorption of devitalized matrix, by *CT* cells, but not by the *PT* cells, was stimulated by parathormone and that calcitonin inhibited this resorption are in keeping with what would be expected for osteoclasts acting *in situ*[10,11]. Our results also show that when both cell types are in contact with matrix there is a small amount of resorption in the absence of exogenous parathormone. The substantially larger release of hydroxyproline from the matrix by the *CT* cells, however, indicates that even in these basal conditions the *CT* cells function in a manner more analogous to osteoclasts *in vivo*. The basis for demineralisation of the matrix by the *PT* cells is not as clear It could be an inherent property of this cell type since osteocytes (probably derived from osteoblasts[12]) have been suspected of actively resorbing mineral[13] Our culture technique facilitates characterisation of the individual isolated cell populations in contact with the physiological matrix in varying conditions. This approach may permit precise characterisation of the molecular processes of bone development and turnover and its control by a variety of hormonal and nutritional factors.

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T-cell-mediated cytotoxicity against herpes simplex virus-infected target cells

THE control of herpes simplex virus (HSV) infection by immunological mechanisms seems to be complex and is poorly understood Neutralising antibodies to HSV plus complement seem to have no effect on the propagation of HSV infection, because HSV spreads to adjacent cells by passing through intercellular bridges[1–5] Anti-HSV antibodies plus complement, however, destroy virus-infected cells, but cannot prevent the spread of HSV, suggesting that the virus must be transferred to neighbouring cells before immune lysis occurs[1,5] Therefore if lymphocyte-mediated cytolytic mechanisms are instrumental in blocking the spread of HSV *in vivo*, they ought to destroy infected cells at a very early stage in the viral maturation cycle, before infectious viral particles are produced. Indeed, effective antibody dependent cell-mediated cytotoxicity (ADCC) has been demonstrated *in vitro* using target cells infected with HSV[4,5]. In this system K-cell-mediated destruction of virus-infected cells takes place very early in the infectious cycle[5]. As *in vivo* protection against HSV infection is predominantly T-cell-dependent[6,7], the *in vivo* spread of HSV infections may be controlled by virus-specific cytotoxic T lymphocytes (CTL). But no virus-specific CTL so far have been detected in the spleen of HSV-infected mice. Using a different experimental approach we have now shown that T lymphocytes of mice undergoing a local HSV infection, when transferred *in vitro*, differentiate into effective CTL. The CTL generated are highly cytotoxic for HSV-infected target cells.

HSV type 1 strain Lennette was propagated in primary rabbit kidney cells as described before[8]. About $1–5 \times 10^5$ plaque-forming units (PFU) of HSV were injected into the hind footpad of CBA/J mice. After 3 d the draining lymph nodes (LN) were isolated and teased, and single-cell suspensions were prepared. The cells were cultured for a further 72 h at 37 °C, a period required to facilitate full differentiation of the sensitised CTL precursors (K. P. *et al.*, to be published). Thereafter, the LN cells were tested for the presence of virus-specific CTL in a 6-h cytotoxicity assay[9] using thioglycollate-induced ^{51}Cr-labelled, HSV-infected CBA macrophages as targets. The syngeneic target cells were infected *in vitro* with 2–5 PFU of HSV per cell 16 h before the ^{51}Cr assay.

Representative results obtained in 17 experiments of this type are shown in Table 1. Accordingly, LN cells draining the site of a local HSV infection *in vivo*, when transferred *in vitro*, differentiated into cytotoxic lymphocytes (CL) with strong lytic activity towards HSV-infected syngeneic macrophages. In contrast, LN cells draining a site which had been injected only with culture medium (control) did not develop cytolytic activity. Pretreatment of CL with anti-Thy 1.2 serum plus complement abrogated their cytotoxic activity (Table 2), strongly suggesting that the CL were T cells. LN cells not removed 3 d after local HSV infection and cultured for 3 d *in vitro*, but obtained *in vivo* 6 d after infection, showed only marginal cytotoxic activity. On the other hand, LN cells draining a local HSV infection until day 10, on transfer *in vitro*, generated effective HSV immune CTL.

Table 1 Lysis of HSV-infected syngeneic macrophages by HSV-induced cytotoxic effector cells

Experiment no.	*In vivo* sensitisation	% Lysis of ^{51}Cr-labelled macrophages: HSV-infected 50 : 1*	HSV-infected 5 : 1	Normal 50 : 1	Normal 5 : 1
	HSV	77	31	11	2
165	Culture medium	−2	1	−3	−1
	HSV	66	26	22	4
179	Culture medium	−4	+1	0	0
	HSV	46	21	42	20
172	Culture medium	−1	0	0	−2

CBA mice received either a given concentration of Lennette strain of type 1 HSV virus in Dulbecco's modified Eagle's medium (DMEM) or DMEM alone by injection in the hind footpad. After 3 d the draining LNs were dissected, single-cell suspensions were prepared and the cells were cultured for additional 72 h in DMEM supplement with 5% foetal calf serum (FCS). The LN cells were then tested for cytotoxicity at various ratios of attacker cells to target cells against ^{51}Cr-labelled syngeneic macrophages[9]. The thioglycollate-induced CBA macrophages were either HSV-infected or non-infected. HSV-infected macrophages were prepared by exposing the cells to Lennette strain type 1 HSV at a multiplicity of infection of 2–5 PFU per cell at 37 °C in DMEM plus 5% FCS for 1 h at 37 °C. The infected macrophages were washed and incubated for additional 15 h at 37 °C. Assay time of the ^{51}Cr cytoxicity test (using 2×10^4 target cells) was 5–6 h. Background lysis of the infected or non-infected macrophages was less than 31%. The percentage lysis data were calculated as before[9]. Of a total of 17 experiments performed, three representative experiments are detailed.

*Ratio of attacker cells to target cells.

Table 2 Effect of anti-Thy 1.2 serum plus complement

Treatment of cytotoxic lymphocytes	% Lysis of HSV-infected CBA macrophages 40 : 1*	4 : 1
None	66	31
Complement alone	70	40
AKR anti-Thy 1.2 serum plus complement	6	3
AKR-serum plus complement	67	38

Cytotoxic lymphocytes towards HSV-infected targets were raised as described in Table 1. The cells were divided into four groups; one group remained without treatment, the others were treated either with complement, anti-Thy 1.2 serum plus complement or with normal AKR serum plus complement. The remaining cells were tested in a 4.5-h ^{51}Cr assay for cytotoxicity towards HSV-infected CBA macrophages (2×10^4) at a cell dilution resulting in the ratio of CL to targets given (for the untreated group). Background lysis of the targets was less than 27%.

*Ratio of CL to target cells.

In four of 17 experiments the CTL generated were almost equally cytotoxic for non-virus-infected, normal syngeneic macrophage targets as for virus-infected cells (see experiment 172, Table 1). This result *a priori* would contradict a possible virus specificity of the CTL generated. In most experiments, however, the CTL generated lysed non-infected syngeneic targets only to a limited extent (experiments 165 and 179; Table 1), so the possibility of the existence of HSV-specific CTL was investigated further.

Because in the above experiments the CTL were assayed for cytotoxic activity in a 6-h ^{51}Cr assay, and because HSV surface antigen was expressed on infected macrophages as early as 2–3 h after infection (K. P. *et al.*, to be published),

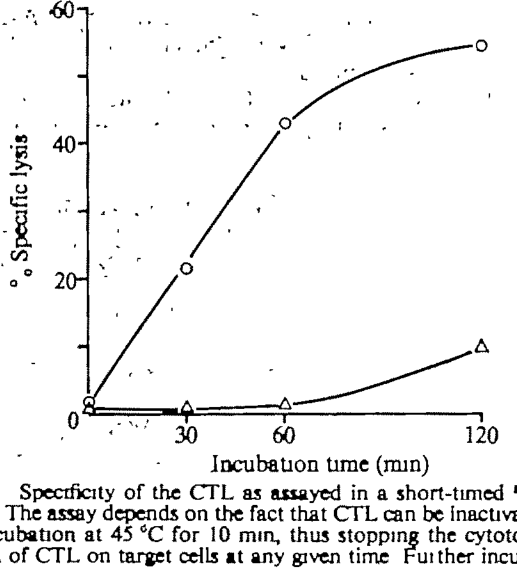

Fig. 1 Specificity of the CTL as assayed in a short-timed ^{51}Cr assay. The assay depends on the fact that CTL can be inactivated by incubation at 45 °C for 10 min, thus stopping the cytotoxic action of CTL on target cells at any given time. Further incubation for 3 h will lead to the manifestation of the previously set cytotoxic action (for details see ref. 10). CBA mice were injected with $1–5 \times 10^5$ PFU HSV into the hind footpad. Three days later the cells of the draining LN were prepared, cultured for additional 72 h *in vitro* and then tested for cytotoxicity towards HSV-infected[1] (○—○) or non-infected[2] (△—△) target cells (2×10^4). Replicates of mixtures of CTL and target cells were set up at a ratio of 50:1 and incubated at 37 °C. At each of the time intervals indicated, triplicates of the two experimental groups were transferred for 10 min to 45 °C, and then incubated for 3 h at 37 °C. Percentage specific lysis was calculated as before[9]. For control 1, cytotoxic activity of CTL to HSV-infected macrophages at 37 °C over 4 h was 62%. For control 2, cytotoxic activity of inactivated CTL (10 min at 45 °C) at 37 °C over 4 h was 2%. Background lysis of the targets was less than 28%. Similar results were obtained in five independent experiments.

it is possible that an infection of 'normal' macrophage targets may take place during the 6-h ^{51}Cr assay. Infectious HSV particles carried over during the ^{51}Cr test from the effector cell population to the 'normal' macrophage targets would thus induce an intra-experimental HSV infection of normal targets, and render them susceptible to the cytolytic action of the CTL generated. We therefore tested for virus-specific cytotoxicity in an experimental system in which the time of contact between CTL and target cells could be reduced to less than 1 h In such assay conditions only targets which had been infected before the onset of the ^{51}Cr assay would be lysed.

The T-cell-mediated cytolytic effector phase can be dissected experimentally into a T-cell-dependent short-timed phase of antigen recognition and lethal hit, and into a T-cell-independent time-consuming phase of cytolysis[9]. It is therefore experimentally possible to reduce the time of interaction between CTL and the respective target cells to less than 1 h[10]. We used this approach in the HSV-system The results (Fig. 1) clearly indicate that a contact time between CTL and HSV-infected targets of less than 1 h was sufficient for strong lysis of the targets More important, the cytolytic activity of the CTL was restricted to HSV-infected targets In these conditions, no lysis of normal targets was detected Further studies showed that the lytic activity of HSV-specific CTL generated with the methodology described is restricted to recognition of HSV antigens in the context of syngeneic histocompatibility antigens (H-2 restriction).

T lymphocytes of mice draining a local site infected with HSV therefore differentiate *in vitro* into CTL In a short-timed cytotoxicity assay these CTL are cytolytic for HSV-infected syngeneic targets Non-infected syngeneic targets are not lysed. *In vitro* HSV-infected syngeneic targets are effectively destroyed by HSV-induced CTL, so such cells may be involved in controlling the *in vivo* spread of HSV infection Because HSV-induced CTL lyse syngeneic targets as early as 2–3 h after infection and because HSV replication in infected cells requires at least 8–12 (ref 5) HSV-induced CTL may be cytotoxic towards infected targets well before viral progeny is produced

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Insulin inhibition of antibody-dependent cytoxicity and insulin receptors in macrophages

INSULIN receptors have been demonstrated on various human white blood cells, including monocytes[1,2] granulocytes[3] and cultured lymphoblastoid cells[4]. Of these, the insulin receptor of the monocyte, a circulating macrophage, has been the most useful in the study of human disease[5-7], although insulin has no known effect on macrophage function We demonstrate here that cultured mouse macrophages[8] and macrophage-containing cell populations from normal mouse spleens both possess specific receptors for insulin with properties indistinguishable from those of the human monocyte, and that insulin, at physiological concentrations, inhibits the ability of these cell populations to lyse antibody-coated red cells (antibody-dependent cell-mediated cytotoxicity or ADCC)

^{125}I-insulin binding to cultured tumour macrophages (P388D$_1$ cell line), mouse spleen cells and human mononuclear cells demonstrated properties which characterise classical insulin receptors[11-14]. The specificity of receptor binding was virtually identical for the three cell types (Fig. 1) Competition curves for ^{125}I-insulin binding by unlabelled porcine insulin revealed 50% inhibition of tracer

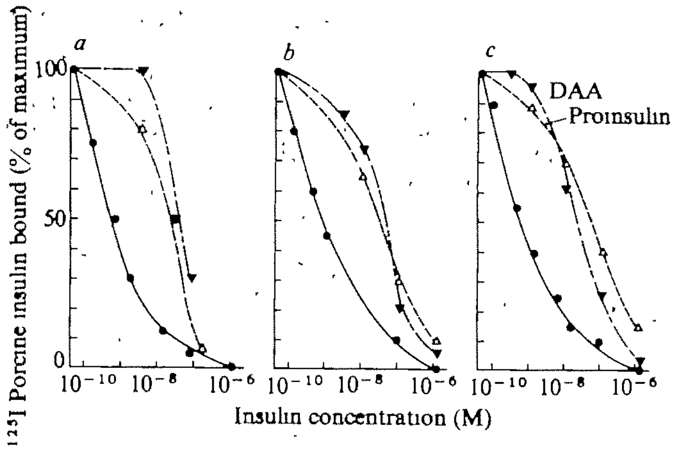

Fig. 1 Inhibition of ^{125}I-insulin binding by insulin analogues in *a*, human mononuclear cells, *b*, mouse spleen cells and *c*, cultured P388D$_1$ macrophages Human mononuclear cells were prepared using Ficoll–Hypaque gradients by Boyum's method[20] The preparations contained approximately 20% monocytes by the criteria of latex bead ingestion, and 80% lymphocytes Spleen cells were prepared from 6–10-week-old, DBA/2 male mice by a modification of the method of Mishell and Dutton[21] P388D$_1$ cells were maintained in Eagle's MEM supplemented with 10% foetal calf serum with fresh culture medium added every 48–72 g and were used for ^{125}I-insulin binding studies approximately 24 h after fresh medium was added Crystalline porcine insulin (Eli Lilly) was labelled with ^{125}I at a specific activity of 130–230 μCi μg^{-1} by a modification of the Hunter and Greenwood method[22-24]. Tracer amounts of ^{125}I-porcine insulin (0 2 ng ml^{-1}) were incubated with 4 × 10^{6} macrophages in 0 5 ml buffer (100 mM HEPES, *p*H 7.8 for P388D$_1$ and spleen cells or *p*H 8.0 for human monocytes) and increasing amounts of either unlabelled porcine insulin (●), unlabelled porcine proinsulin (△), or unlabelled DAA-insulin (▲) After 3 h of incubation (at 15 C for P388D$_1$ and spleen cells or 22 C for human monocytes), replicate 200-μl samples were transferred to microfuge tubes and centrifuged for 1 min. The resulting supernatant fluid was aspirated, the cell pellet was excised and the cell-associated radioactivity was counted Nonspecific binding (radioactivity bound to cells in the presence of porcine insulin, 100 μg ml^{-1}) was subtracted from total binding to yield specific binding. Maximum specific binding (100% value) for the human monocytes was 6%, for the mouse spleen macrophages 8% and for the cultured macrophages 13%

binding at 10^{-8} M for all three cell populations. Porcine proinsulin and desalanine desasparagine insulin (DAA-insulin), which have 1–5% of the biological activity of porcine insulin, were 1–5% as potent as porcine insulin in competing for ^{125}I-insulin binding to the insulin receptors of these cells (Fig 1). Thus, the receptors on all three cell types showed similar inhibition of insulin binding by insulin analogues with the receptor potency of the insulin analogue proportional to its biological activity.

In addition to specificity of binding, several physicochemical properties of the insulin receptors in these three cell populations were similar to more classical insulin receptors ^{125}I-insulin binding showed a sharp optimum at pH 7 8–8.0 The amount of ^{125}I-insulin bound in steady-state conditions increased with temperature in the order 4, 15, 22 and 37 °C. Both the association rate and the dissociation rate of ^{125}I-insulin binding increased with temperature, with the increase in the dissociation rate being greater than the increase in the association rate. Equilibrium analyses of insulin binding to the three populations of cells gave curvilinear Scatchard plots, and kinetic studies demonstrated that excess unlabelled insulin accelerated the dissociation of ^{125}I-insulin that had been bound previously to receptor These latter two findings indicate site–site interactions among insulin receptors of the negatively cooperative type[13]

Previous studies have shown that macrophages may act as effector cells in *in vitro*, antibody-dependent cytotoxicity assays[14–16]. We found that insulin inhibited ADCC in both the tumour macrophages (P388D$_1$ cell line) and the mixed cell population prepared from mouse spleens (Fig 2*a*, Table 1). Inhibition by insulin was most pronounced at low effector(macrophage)-to-target cell ratios (Fig. 2*b*, Table 1) At an effector-to-target cell ratio of 1 25 : 1, insulin at 10^{-8} M inhibited P388D$_1$-induced lysis by more than 70% and 10^{-10} M insulin inhibited lysis by 25% With the mouse spleen cells, at ratios of 2 5 : 1, 10^{-8} M insulin inhibited lysis by 29% and 10^{-10} M insulin inhibited lysis by 15%. In P388D$_1$ cells, the inhibition of ADCC by insulin was dose-related for a wide range of insulin concentrations (Fig. 3). In both systems, insulin concentrations as low as 10^{-10} M, which is clearly within the physiological range, produced a significant decrease in cell lysis. This inhibitory effect of insulin was reproducible in several experiments, the data in Figs 2 and 3 and Table 1 being representative.

In P388D$_1$ cells the inhibitory effect of insulin was exerted through the macrophages and not through insulin-induced alterations in target red cells When the P388D$_1$ cells were preincubated with insulin and then washed before exposure to target cells, the effect of insulin on ADCC was still observed If the target cells were first preincubated with insulin, thoroughly washed and then exposed to P388D$_1$ cells, however, ^{51}Cr release was not inhibited and was similar to that obtained in the absence of insulin (data not shown).

Both the mouse spleen cells and human mononuclear cells represent mixed cell populations. In the cell population prepared from mouse spleens, both insulin binding and cytotoxic activity seem to be due to the macrophages. When these spleen cells were depleted of macrophages by passage through a Sephadex G-10 column[17], insulin binding was decreased by 75% and ADCC was abolished (Table 1, depleted fraction) Moreover, when macrophage-enriched cells were eluted from the column before use, both cytotoxic activity and ^{125}I-insulin binding were restored (Table 1, enriched fraction). We previously found similar ^{125}I-insulin-binding results in enrichment and depletion experiments with the mixed mononuclear cell preparations from human blood; coupled with radioautography, these studies established that monocytes and not lymphocytes account for the observed insulin binding in this mixed cell preparation[3].

Macrophages from normal mouse spleens and cultured tumour macrophages (P388D$_1$ cell line) thus possess insulin receptors indistinguishable from those of the circulating human monocyte and other mammalian tissues. Furthermore, physiological concentrations of insulin alter a specific immunological function, antibody-dependent cell-mediated cytotoxicity, in both the spleen and tumour cells. In the spleen cells, insulin maximally inhibited ADCC by 30%, while in the more homogenous P388D$_1$ cells, insulin inhibited ADCC by more than 70%. The potency of insulin in the two cell systems may differ because spleen cells are a mixed cell population; multiple effector cells could exist in this preparation, with insulin acting on only a minority of the effector cells.

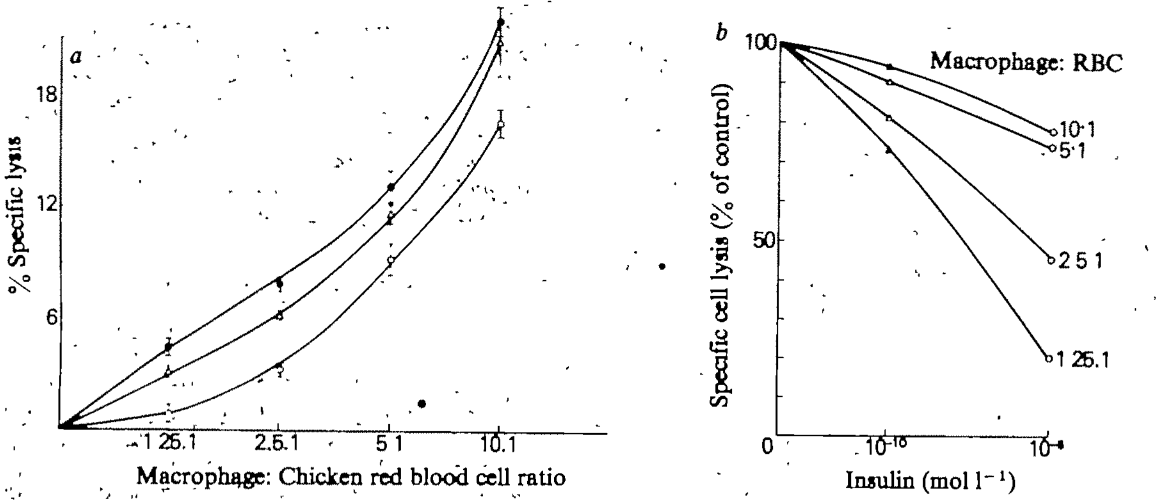

Fig. 2 *a*, The effect of insulin on antibody-dependent cytotoxicity in cultured tumour macrophages (P388D$_1$ cell line) Chicken red blood cells were labelled with ^{51}Cr and coated with anti-trinitrophenyl antibodies as previously described[15] The effector cells (P388D$_1$ cells) were preincubated with or without insulin at 37 C for 30 min Various concentrations of effector cells were then added to 5 10^4 target red cells in a final volume of 1 ml; and incubated at 37 C for 2 h. At the end of the incubation period the reaction mixture was centrifuged at 700*g* for 10 min at 4 °C The resulting supernatant was counted and specific cell lysis was calculated using the formula: percentage specific lysis $= (E - S)/(T - S) \times 100$, where E = counts of experimental group, S = counts from supernatant fluid of assay tubes containing only ^{51}Cr-labelled red cells (spontaneous release) and T = total radioactivity in assay tube. Spontaneous release in this experiment was 1.1%. Details of this ADCC assay have been previously described[15,18]. The percentage specific lysis by effector cells preincubated with buffer alone (●), with 10^{-8} M insulin ( ) and with 10^{-10} M insulin (△) are plotted for different ratios of effector:target cells All points represent the mean ± s.e. of triplicate samples. *b*, Inhibition of antibody-dependent cytotoxicity by insulin plotted as a function of effector–target cell ratios, which varied from 10:1 to 1:25·1. Lysis, calculated as percentage of buffer control at comparable effector–target cell ratio, is plotted as a function of insulin concentration.

Table 1 ADCC and ^{125}I-insulin binding in spleen cells from normal DBA/2 mice

ADCC by mouse spleen cells

E:T*	[Insulin] (M)	% Specific lysis†	% Inhibition of lysis‡	P
10:1	—	45 9±0.5		
10:1	10^{-10}	43.9±0.9	4.3	< 0.05
10:1	10^{-8}	41.9±0.8	8.7	< 0.01
5:1	—	21.9±1.6		
5:1	10^{-10}	18.6±0.6	15.0	< 0.05
5:1	10^{-8}	15.5±1.1	29.2	< 0.025
2.5:1	—	9.9±0.4		
2.5:1	10^{-10}	8.3±0 4	16.1	$< 0 025$
2.5:1	10^{-8}	7.6±0.2	23.2	< 0.025

Effect of macrophage enrichment and depletion on ADCC

	E:T	% Specific lysis	% Control	% ^{125}I-insulin binding per 30 × 10^6 cells
Initial cell preparation	5:1	20.0±0.3	100	4.1
	2.5:1	10.2±0.3	100	
Depleted preparation	5:1	0.9±0 1	4.5	1.0
	2.5:1	0.6±0.1	6.0	
Enriched (adherent preparation)	5:1	21 1±0.5	106	6 8
	2.5:1	10.7±0 2	104	

* Various concentrations of DBA/2 spleen cells (E) incubated with 2 × 10^5 target cells (T).
† Specific lysis calculated as described in Fig. 2. Spontaneous release in this assay was 7.3%.
‡ Calculated as percentage of control tubes without insulin.

The mechanism by which insulin alters macrophage-mediated ADCC is unknown. Rhodes has shown that insulin, at high concentrations (5×10^{-8} M) and over relatively long periods (72 h), decreases the number of F$_c$ receptors on peritoneal macrophages[18]. The initial step in ADCC involves recognition by the macrophage F$_c$ receptors of the F$_c$ portion of the antibody which coats the target cell, so alterations of macrophage F$_c$ receptors could decrease cell lysis. Whether insulin, at lower concentrations and over the shorter course of our experiments, could decrease the number or affinity of macrophage F$_c$ receptors is unknown.

Strom[19] has shown that physiological concentrations of insulin increase the cytotoxic potential of 'killer' T cells in a direct, lymphocyte-mediated cytotoxicity assay. Taken together with our findings, these observations suggest that insulin can alter the immune system at multiple levels. The *in vivo* significance of our findings, and the potential role of insulin in immune function, however, remain speculative. We suggest that the cell populations described in this report offer unique model systems for studying the interrelations between insulin receptors and an insulin-mediated biological effect on immune function.

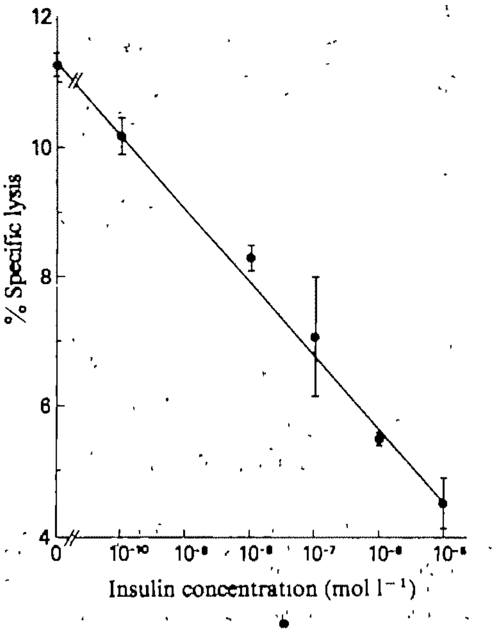

Fig. 3 Dose-response effect of insulin on antibody-dependent cytotoxicity in the cultured tumour macrophage. P388D$_1$ tumour cells were preincubated with various concentrations of insulin then assayed at an effector (macrophage)–target cell ratio of 5:1. Details of the assay are described in the legend to Fig. 2. Each point represents the mean ± s.e. of triplicate samples.

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Effect of flavone inhibitors of transport ATPases on histamine secretion from rat mast cells

INTERACTION of multivalent ligands with receptors (immunoglobulin E, IgE) on the surface of the mast cell stimulates the calcium-dependent degranulation and secretion of histamine[1,2]. Secretion is also initiated by application of the calcium carrier substance (ionophore) A23187 (ref 3), and so the effective common stimulus to this cell (and many others) is the entry of Ca^{2+} ions into the cytosol. The mechanism of the normal ligand-induced calcium entry process is not well understood[4]. Certain flavones, notably quercetin, interfere with the activity of membrane transport adenosine triphosphatases (ATPases) including the calcium-dependent ATPase which is associated with exclusion of calcium from the cytosol of cells. They are not simple ATPase inhibitors but probably act by increasing the efficiency of ion translocation, thus reducing the consumption of ATP (refs 5, 6). Also, the flavones (2-phenyl cromones) have some structural resemblance to the widely used anti-allergic drug cromoglycate whose activity may be expressed at the calcium entry pathway[7]. We examine here the effect of these substances on histamine secretion from mast cells.

The effects of six flavones on antigen-induced histamine secretion are shown in Table 1. While two of these have little effect even at 100 μM, fisetin, quercetin, myricetin and kaempferol inhibit strongly at 33 μM. Fisetin, quercetin and myricetin interfere with transport ATPases while neither of the non-inhibitory flavones is active in this respect[8].

Since ionophore-mediated release bypasses the normal calcium entry pathway regulated by multivalent ligands[1], it distinguishes between inhibitors which act on or before calcium entry and those which affect a later stage in the secretory process[4,10]. Thus, whereas quercetin inhibits release stimulated by antigen and concanavalin A, which bind to IgE (refs 1, 9) (Fig. 1), release induced by ionophore A23187 is only slightly affected. It is unlikely that this latter effect arises from an action of the quercetin on the cells because (1) mixtures of A23187 and quercetin form visible precipitates; (2) the fluorescence emission spectrum of A23187 is red shifted in the presence of quercetin; (3) the permeability of liposomes rendered permeable to Ca^{2+} and Fe^{2+} with A23187 is reduced by the addition of quercetin.

Table 1 Effect of flavones (at 33 and 100 μM) on antigen-induced histamine secretion from rat mast cells and on Ca^{2+}-dependent ATPase activity of rabbit skeletal muscle sarcoplasmic reticulum

	Histamine secretion		Ca^{2+}-dependent ATPase activity	
	33 μM	100 μM	33 μM	100 μM
Fisetin	0.31	0.00	0.91	0.57
Kaempferol	0.59	0.24	0.64	0.30
Morin	0.99	0.82	0.95	0.74
Quercetin	0.37	0.00	0.69	0.37
Myricetin	0.42	0.26	0.74	0.37
Rutin	1.00	0.75	0.99	0.91

Results are expressed as a fraction of the control value obtained in the absence of flavone. Peritoneal mast cells purified to >80% homogeneity[19] were obtained from rats sensitised to egg white protein. Cells were preincubated at 37 for 5 min in Tyrode's solution containing 10 μM phosphatidyl serine before addition of the releasing agent and flavones. (The phosphatidyl serine was added to enhance the degree of ligand mediated secretion[20]). After 2 min the reaction was ended by addition of ice-cold saline, and histamine in the supernatant was measured fluorimetrically[21]. Any interference due to the flavones was compensated for by using appropriate blanks and standards. The Ca^{2+}-ATPase activity of rabbit muscle sarcoplasmic reticulum[22] was determined by measurement of the rate of H^+ release during ATP hydrolysis in a solution containing 250 mM sucrose, 100 mM KCl, 5 mM $MgCl_2$, 8.2 mM potassium oxalate, 15 mM sodium citrate, 2 mM ATP, 10 mM Tris buffer. The pH was adjusted to 7.5 and free calcium concentration was brought to 10 μM by addition of 0.4 mM $CaCl_2$. The reaction was initiated by addition of sarcoplasmic reticulum membranes, giving a final concentration of about 0.12 mg protein per ml. The specific activity of the preparation was about 1 μmol mg^{-1} min^{-1} at room temperature. Less than 10% activity was obtained in the absence of calcium.

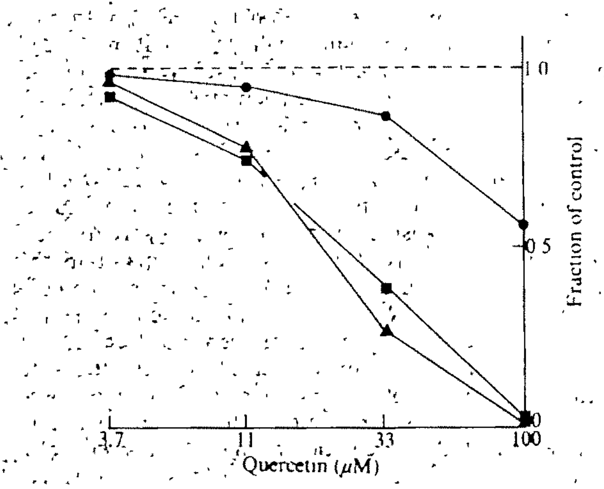

Fig. 1 Effect of quercetin on histamine release from mast cells stimulated with releasing agents. Methods are as in the legend to Table 1 except that the ionophore A23187, which is fluorescent, was extracted into hexane before histamine assay. Results are expressed as a fraction of control obtained in the absence of flavone. Mean s.e. ±0.04. ●, A23187 (5 μM) seven experiments; ■, antigen (10 μg ml^{-1} egg white protein) seven experiments; ▲, concanavalin A (10 μg ml^{-1}) five experiments.

Our results support the idea that the effect of quercetin on ligand-stimulated cells involves blockade of the normal calcium entry pathway. A similar argument explains the action of the phosphodiesterase inhibitors theophylline and dibutyryl cyclic AMP on mast cell secretion[7], and dibutyryl cyclic AMP was shown to prevent concanavalin-A-induced $^{45}Ca^{2+}$ uptake by T lymphocytes[11]. Inhibition of mast cell secretion by cromoglycate could be mediated by a similar mechanism[12]. Quercetin has negligible activity as an inhibitor of the cyclic AMP phosphodiesterase of human lung (J. Hare and I. F. Skidmore, personal communication) and unlike theophylline and dibutyryl cyclic AMP the effect of quercetin is manifest when it is added to the cells simultaneously with the releasing ligand. In this respect, it resembles cromoglycate, but, unlike quercetin, the effect of cromoglycate is of short duration[13], and is much less active on cells treated with phosphatidyl serine.

Our results indicate that we are dealing with a novel regulator of the calcium pathway.

At the concentrations used to inhibit mast cell secretion the activity of the flavones seems to be restricted to membrane transport ATPases[14,15]. The effective flavones alter the activity of several ATPases including plasma membrane $(Na^+ + K^+)$ ATPase of heart muscle[16], mitochondrial ATPase[15] and the Ca^{2+} ATPase of chloroplasts[17,18]. The effect of quercetin on histamine release is unlikely to be due to inhibition of mitochondrial ATPase, as this should affect secretion induced by A23187 as well. It is also unlikely to be due to inhibition of the $(Na^+ + K^+)$ ATPase since ouabain even at 1 mM had no effect on the antigen-induced process. The secretory event involves Ca^{2+} movements, so it is most likely that the effect of quercetin on the abolition of secretion from ligand-stimulated cells is directed at the Ca^{2+} ATPase in the plasma membrane.

We therefore tested the flavones as inhibitors of rabbit sarcoplasmic reticulum Ca^{2+} ATPase (Table 1). It would be desirable to compare the inhibition of the mast cell secretory reaction with Ca^{2+} ATPase from the same source, but as it has been claimed[19] that the major Ca^{2+}-ATPase of the mast cell is reactive towards externally supplied ATP it is doubtful whether this enzyme is involved in Ca^{2+} transport. Even the association of this enzyme with the mast cell is open to doubt since the ecto-ATPase of 'purified' mast cell preparations is largely accounted for by the presence of contaminating macrophages[20]. We have therefore preferred to work with the Ca^{2+}-ATPase of sarcoplasmic reticulum which has a well-defined Ca^{2+} transport function. All of the compounds which inhibit mast cell histamine release also inhibit Ca^{2+} ATPase while morin and rutin are hardly inhibitory in either system.

Racker suggested that the transport ATPases of cell membranes and the associated ion flux pathways are separate structural entities, which when coupled together constitute the ATP dependent ion pumps[6]. In certain tumour cell lines the ion transport functions are apparently uncoupled from the ATPase functions[8] and to maintain the intracellular ionic milieu, ATP consumption rises. The consequent availability of ADP results in the characteristic enhancement of glycolysis and depressed *p*H (ref. 22) of some tumour tissues (Warburg effect). When treated with quercetin the efficiency of the transport ATPase is restored and the glycolytic rate declines[8] although quercetin is not an inhibitor of any of the glycolytic enzymes[23]. Quercetin also improved the Ca^{2+}-to-ATP ratio in reconstituted sarcoplasmic reticulum preparations[8].

Our results suggest that some flavones which affect the efficiency of membrane transport ATPases also prevent Ca^{2+} influx into ligand-stimulated mast cells, raising the possibility that the inhibitor is active at a common locus in both systems. We suggest that Ca^{2+} movements, that is, the efflux due to Ca^{2+} pumping, and influx which follows cross linking of surface receptors, share a common pathway. In the resting cell, the pathway is tightly coupled to the ATPase, but when the cells are treated with cross-linking ligands, the ATPase becomes uncoupled transiently from the flux pathway and there is a net movement of Ca^{2+} down its concentration gradient into the cell. Quercetin could prevent the ligand-induced uncoupling of the ATPase. The inhibitory effect of cyclic AMP on ligand-induced histamine release could also be exerted at the Ca^{2+} ATPase as cyclic AMP is known to modulate the effect of this enzyme[24].

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Membrane defect affecting water permeability in human epilepsy

MCQUARRIE wrote that a disturbance in water balance perhaps affecting the central nervous system more specifically seems to be closely identified with the aetiology of epilepsy[1]. McQuarrie and Teglbjaerg[2] studied water balance in relation to seizure frequency, establishing the ictogenic effect of excessive water ingestion, and the therapeutic value of dehydration. Schneider[3] has reported that exacerbation of *petit mal* in children and adults is associated with reduction in urinary volume, and that clinical remission coincidences with an increase in water excretion. Reynolds[4] has demonstrated disturbances in the whole body distribution of water in epilepsy, with a magnitude proportional to the frequency of attacks, concluding that the changes in body water and sodium are linked in some way to the aetiology of the disease. Wender and Strzyzewski[5] have shown that water loading in epileptics produces seizures only in cases where there is also a marked increase in the water content of erythrocytes. There is thus abundant evidence linking epilepsy with water metabolism. Furthermore, diuretics, such as acetazolamid, are used in the therapy of epilepsies[6]. But the mechanism of the disturbance of water metabolism at the subcellular and molecular level has not been considered. We report here an investigation of water diffusion through erythrocyte membranes in epileptic children, made using a nuclear magnetic resonance (NMR) technique[7]. We found an abnormally low permeability in membranes from epileptics and suggest that a generalised membrane defect affecting water permeability is responsible for the disturbances of water metabolism in human epilepsy.

The NMR method has been applied to the study of water transfer across nerve cell membranes by Fritz and Swift[8]. The method involves addition of a paramagnetic solution ($MnCl_2$) to the plasma and measurement of the spin–spin relaxation time (T_2) of the erythrocyte water proton. The spin–spin relaxation time of water inside the isolated erythrocytes is about 140 ms and is much longer than the time required for water to penetrate the membrane (the water exchange time, T_{ae}), which is about 10 ms. If the relaxation time in plasma is made much shorter than the exchange time by adding the paramagnetic ion Mn^{2+}), the observed relaxation time of the erythrocyte (T_{2b}) is dominated by the exchange process through the membrane. T_{2b} is evaluated from a logarithmic plot of the nuclear spin echo as a function of the time between radiofrequency pulses[9]. T_{ae} is calculated as described by Conlon and Outhred[7]. The value of T_{ae} is inversely related to the water permeability of erythrocytes.

Table 1 Clinical features and therapy of epileptic patients

Patient	Age (yrs)	Sex	Epilepsy			Drugs and duration of treatment (yr)
			Classification	Severity	Age of onset (yr)	
1 F.L.	2	F	Idiopathic GM	*A*	0.1	Phb,Dph (2)
2 M.N.	5	M	Idiopathic GM	*A*	3	Phb,Dph (2)
3 B.C.	7	F	Idiopathic GM	*C*	1	Phb,Dph (6)
4 M.V.	9	M	Idiopathic GM	*A*	7	Phb,Dph (2)
5 P.A.	12	F	Idiopathic GM	*A*	8	—
6 B.I.	11	M	Idiopathic GM	*A*	10	Phb,Dph (1)
7 M.V.	10	M	Idiopathic GM	*A*	7	Phb,Dph (3)
8 M.C.	10	F	Idiopathic GM	*A*	6	Phb (4)
9 P.N.	12	M	Idiopathic GM + PM	*A*	9	—
10 P.P.	12	F	Idiopathic PM	*A*	6	Phb (3)
11 M.C.	14	M	Idiopathic GM	*A*	4	Phb,Dph (10)
12 G.A.	17	F	Idiopathic GM	*B*	15	Phb,Dph (2)
13 S.N.	15	F	Focal TL	*A*	13	Phb (1)
14 L.I.	16	M	Focal TL	*A*	15	—
15 B.M.	0.8	M	Focal GM	*A*	0.4	Phb (0.4)
16 B.A.	1	M	Focal GM	*A*	0.5	Phb (0.5)
17 G.V.	1	F	Focal M	*B*	0.1	—
18 C.C.	5	M	Focal GM (Gaucher's disease)	*A*	4	Phb,Dph (1)
19 N.H.	3	M	Focal GM	*A*	2.7	—
20 N.I.	5	F	Focal GM	*C*	2	Phb,Dph (3)
21 M.D.	6	M	Focal M	*A*	4	Phb,Dph (2)
22 L.V.	9	F	Focal M	*C*	9	Phb,Dph (0.3)
23 B.S.	10	M	Focal GM	*A*	8	Phb (2)
24 B.L.	10	M	Focal GM	*A*	9	Phb,Dph (1)

GM, *grand mal;* PM, *petit mal;* M, minor attacks; TL, temporal lobe; Phb, phenobarbital and Dph, diphenylhydantoin. Severity is graded as *A*, severe (attacks either daily or on most days of the week); *C*, mild (attacks less than once a month); the remainder were classified as moderate, *B*.

Table 2 Increases of water exchange time through erythrocyte membranes in epileptic patients

Patient	Blood sample no.	Temperature (°C)	Method	Free Mn^{2+} concentration in plasma (mM)	ΔT_{ae} %	Mean* ΔT_{ae}(%)
1 F.L.	1	37	CPMG	19.5	21.8	21.7
	2	37	CPMG	19.5	23.0	
	3	37	CPMG	19.5	19.8	
	4	37	CPMG	19.5	22.0	
	5	37	CPMG	19.5	21.9	
2 M.N.	1	20	90°*t* 180°	9.5	14.5	15.2
	2	20	90°*t* 180°	9.5	16.0	
3 B.C.	1	20	90°*t* 180°	9.5	12.5	11.5
	2	20	90°*t* 180°	9.5	10.5	
4 M.V.	1	20	90°*t* 180°	9.5	30.4	29.9
	2	37	90°*t* 180°	19.5	29.5	
5 F.A.		37	CPMG	19.5	35.6	36.3
	2	37	CPMG	19.5	37.0	
6 B.I.	1	37	90°*t* 180°	19.5	20.3	
7 M.V.	1	20	90°*t* 180°	9.5	32.0	
8 M.C.	1	20	90°*t* 180°	9.5	17.5	16.3
	2	37	CPMG	19.5	15.5	
9 P.N.	1	37	CPMG	19.5	25.0	
10 P.P.-	1	37	CPMG	19.5	32.0	
11 M.C.	1	37	CPMG	19.5	19.5	13.3
12 G.A.	1	37	90°*t* 180°	19.5	10.5	
13 S.N.	1	20	90°*t* 180°	9.5	4.6	
14 L.I.	1	20	90°*t* 180°	9.5	14.4	
15 B.M.	1	20	90°*t* 180°	9.5	23.4	
16 B.A.	1	37	90°*t* 180°	19.5	34.6	
17 G.V.	1	20	90°*t* 180°	9.5	4.6	
18 C.C.	1	20	90°*t* 180°	9.5	46.0	47.0
	2	20	90°*t* 180°	9.5	48.0	
19 N.H.	1	20	90°*t* 180°	9.5	5.7	
20 N.I	1	37	CPMG	19.5	12.0	
21 M.D.	1	37	CPMG	19.5	7.9	
22 L.V.	1	20	90°*t* 180°	9.5	18.7	
23 B.S.	1	20	90°*t* 180°	9.5	21.4	
24 B.L.	1	20	90°*t* 180°	9.5	15.0	

Mean ± s.e.m. 19.8 ± 2.2%.

The sample was made by mixing 1 ml of heparinised blood and 0.5 ml of 20 or 40 mM $MnCl_2$ solution in 0.15 M NaCl. NMR measurements were performed with a Bruker–Physik SXP spectrometer, at 90 MHz. The 90° pulse had a width of 5.5 µs. The spin–spin relaxation time was measured by the 90°*t* 180° method and the CPMG method with a pulse sequence spacing of 0.8 ms. Phase detection was used in both cases, and the field was stabilised with the BSN 15 external field stabiliser. The temperature unit of the spectrometer was adjusted to ± 0.5 °C. Measurements were made within several min of preparation of the sample. Blood samples from the epileptic and control children were handled simultaneously to assure comparable conditions. The results are expressed as percentage increase of the water exchange time (ΔT_{ae} %) in epileptic subjects, the reference being the normal blood. The reference values of T_{ae} for the normal blood were for the 90°*t* 180° method: 11.1 ms at 20 °C and 9.5 mM Mn^{2+} in plasma, and 8.2 ms at 37 °C and 19.5 mM Mn^{2+} in plasma. For the CPMG method T_{ae} = 8.7 ms at 37 °C and 19.5 mM Mn^{2+} in plasma.

Patients were 24 epileptic children aged 1–17 yr; their main clinical features are summarised in Table 1. An equal number of children aged 2–16 yr formed the control group.

Table 2 shows that in all epileptic children the exchange time of water through the red blood cell membranes was longer than in control subjects. The difference between T_{ae} in epileptic patients and controls was always positive, although measurements were made at different temperatures and concentrations of Mn^{2+}, and two methods were used to measure spin–spin relaxation time—the 90° *t* 180° method[7] and the Carr–Purcell–Meiboom–Gill (CPMG) method (which eliminates errors due to the inhomogeneity of the magnetic field and the molecular diffusion in field gradients[9]). It seems that the water permeability of erythrocyte membrane in epilepsy is less than in the normal situation. There were no significant differences in T_{ae} values between idiopathic and focal epileptics. High values of T_{ae} were noted in patients who suffered from attacks every day and in whom the attacks were poorly controlled by anticonvulsant drugs. But the value of T_{ae} during the attack was not higher than in the *interictal* period. This indicates that the low water permeability of erythrocytes in epilepsy is not transient but represents a permanent alteration. The abnormal water permeability was not related to the anticonvulsant therapy as it was noted in both untreated and treated patients.

Possible explanations for the observed difference in water proton transfer across the cell membranes in epileptics and normal subjects are an alteration of the erythrocyte permeability affecting water diffusion, and osmotic changes following the addition of the 0.15 M NaCl solution to the blood sample. Any osmotic changes would occur very quickly after mixture of the blood with the physiological saline solution. Consequently, such changes could not influence NMR measurements made several min after the blood was mixed with 0.15 M NaCl. Slow osmotic changes can be excluded because exchange time values remained unchanged for hours, both for normal subjects and epileptics. Alteration of erythrocyte permeability affecting water diffusion is thus the most likely explanation for our finding.

Abnormalities in functions of erythrocyte membranes are considered to indicate a generalised membrane defect[10,11]. Within certain limits a close qualitative and quantitative analogy is possible between the stability of the membrane of the erythrocyte and that of the neurone[12,13]. Fritz and Swift[8] have shown (by the NMR method we used) that for the frog nerve the exchange rate of the water protons between the intra- and extracellular environments is lower for the depolarised nerve than for the polarised nerve. That is, the water permeability of the membrane is smaller in the depolarised state than in the resting state.

The polarisation of the membrane is due to the differential distribution of Na and K across the excitable neuronal membranes, maintained by the Na–K-activated ATPase[14]. It has been suggested[15] that the final common expression of epileptogenicity is an impairment of operation of the Na and K transport systems in epileptogenic neurones, which creates the characteristic instability of the excitable membrane. Harris[16] has suggested that changes in distribution or transport of Na^+ and K^+ may not be due only to effects on the energy state or on ATPase activity, but may be a reflection of a generalised defect of membrane permeability.

We suggest that decreased water permeability in erythrocytes of epileptics may reflect a membrane defect in all tissues and may be an expression of the individual predisposition to epilepsy; it might be of particular importance in the nervous system. Further studies on erythrocyte membranes in epilepsy may give clues to the understanding of the membrane defect in molecular terms. The erythrocyte may be a useful model for testing *in vivo* the possible effectiveness of treatment with anticonvulsant drugs in individual patients, or for testing new anticonvulsant drugs.

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New human embryonic antigen and its presence in some adenocarcinomas

IMMUNODIFFUSION methods have identified several embryonic antigens in tumour extracts or in sera of patients with primary cancer of the liver[1–3], colonic adenocarcinoma[4–6], testicular and ovarian teratoblastomas[7,8], carcinoma of the pancreas[9], chorionepithelioma of the uterus[10] and testicular chorioncarcinoma[11], nephroblastoma[12] and adenocarcinoma of the ovary[13,14]. We report here the use of monospecific antisera to identify a new embryonic antigen in human foetal sera and in some adenocarcinoma extracts.

Antisera against extracts of human ovarian adenocarcinomas were prepared in rabbits[13]. After absorbtion by human pooled plasma (20–40 mg ml^{-1}) and pooled extracts

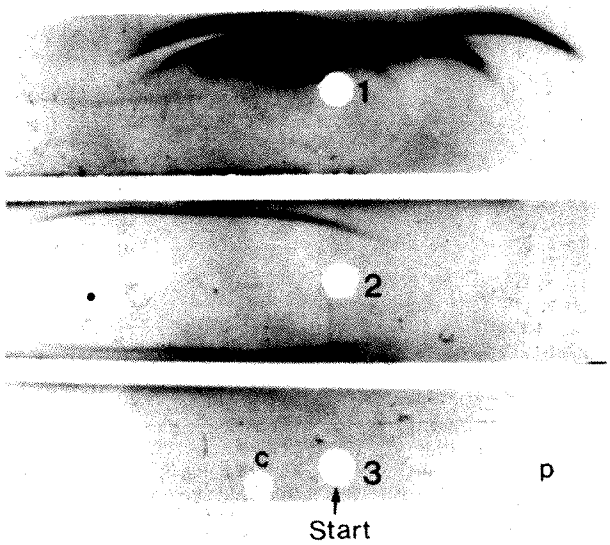

Fig. 1 Gel immunoelectrophoresis of human foetal and adult sera. Precipitation lines were developed with antiserum against human serum proteins[4] and with adsorbed antiserum against ovarian adenocarcinoma extract[2]. Sera of: 1,3, human pregnancy sera; 2, foetus of 20 weeks. Zone of migration: c, colour T-1824; p, pyronine.

Table 1 Immunodiffusion of absorbed antisera against various sera and tissue homogenates

Sample	No. of samples examined	Results of gel diffusion analysis No. positive	No. negative	Titre
Human serum of				
Foetuses of 20–30 wk	20	20	0	1:8–1:32
Foetuses of 30–40 wk	11	11	0	1:2–1:8
Newborns of 30–40 wk	20	20	0	1:1–1:2
Adult donors (male and female)	50	0	50	—
Tissue homogenates of				
Ovarian adenocarcinoma	26	13	13	1:1
Colonic adenocarcinoma	17	7	10	1:1
Carcinoma of stomach	16	3	13	1:1
Uterine carcinomas	9	0	9	—
Lung carcinoma	5	0	5	—
Miscellaneous	10	0	10	—
Normal adult, various organs*	36	0	36	—

*Included ovary, testis, liver, spleen, pancreas, kidney, gastrointestinal tract, brain and muscle tissue.

of normal tissues (40–60 mg ml^{-1}) the antisera were used for immunoelectrophoresis[15] and immunodiffusion in agar[16]. The newly discovered antigen migrated with α-globulin and prealbumin in immunoelectrophoresis (Fig. 1). Similar electrophoretic fractions are present in all human embryonic sera. Immunodiffusion analysis revealed analogous antigen in sera of newborns as well as in some individual extracts of ovarian and colonic adenocarcinomas (Fig. 2) but not in adult sera or in other normal and malignant tissues. These data are summarised in Table 1.

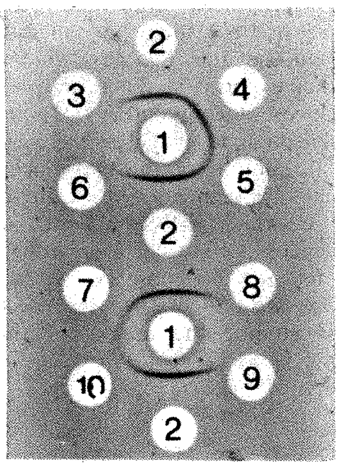

Fig. 2 Gel double diffusion with standard test system: 1, monospecific antiserum; 2, standard antigen. Human serum of 3, adult donor: 4, foetus 20 weeks; 7, newborn. Homogenates of 5,6, ovarian adenocarcinomas; 8, hypernephroid tumours; 9,10, colon carcinomas.

Comparative immunodiffusion showed that the reported embryonic antigen was not identical to α-foetoprotein[17], carcinoembryonic antigen[4], bovine fetuin[18], or pregnancy-specific β-1-globulin[10].

The results suggest that the newly identified antigen is a serum protein secreted during embryonic development, and can be synthesised in adults with adenocarcinomas. The physiological role and cellular localisation of this antigen are not known.

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Reconstitution in phospholipid vesicles of a glucose transport system from pig small intestine

Absorption of monosaccharides from the small intestine in mammals occurs by several different processes. The major barrier to absorption is the brush border membrane of the epithelial cells across which glucose is transported against its concentration gradient in a sodium-coupled transport system. We describe here some properties of monosaccharide transport from phospholipid vesicles into which brush border proteins obtained from neonatal pig small intestine were incorporated. The system transported glucose, but not fructose more rapidly in the presence of a sodium gradient; it transported D-glucose more rapidly than L-glucose and efflux was reduced by phlorizin.

Small intestines were removed from unfed newborn pigs from the Babraham herd. All biochemical procedures were performed in a cold room at 5 °C. Preparations of small intestinal brush border membranes were made by a modification of the method of Porteous[1]; purity was checked by phase-contrast microscopy. There was no reaction to the diphenylamine reagent test for DNA in brush-border samples. Table 1 shows the results of enzymatic assays on subcellular fractions. Although there was little mitochondrial contamination there was evidence of acid phosphatase release from lysosomes. The preparation contained some baso-lateral membranes. Brush border proteins were either immediately incorporated into phospholipid vesicles, or stored deep frozen for up to 2 months before use, with small loss in glucose-transporting activity.

Phospholipid vesicles were prepared from purified soya bean phospholipids using cholic acid, the modification by

Table 1 Enzymatic activities in small intestines of newborn pigs

Enzyme	Crude homogenate	Mitochondrial fraction	Brush-border fraction
Monoamine oxidase[2] (nmol tyramine oxidised h^{-1} mg protein^{-1})	200.9	318.6	189.2
Succinic dehydrogenase[3] (μmol 2–6 dichlorophenolindophenol oxidised h^{-1} mg protein^{-1})	7.04	31.4	10.5
Acid phosphatase[4] (μmol *p*-nitrophenylphosphate metabolised h^{-1} mg protein^{-1})	212.8	976.2	1872.2
Sucrase[5] (μmol metabolised h^{-1} mg protein^{-1})	—	—	< 50

Warren *et al.*[6] of Racker's[7] method. Vesicles containing brush border protein were prepared in sodium–sucrose buffer (*p*H 8.0) containing 250 mM sucrose, 1 M NaCl, 50 mM NaH_2PO_4 and 2 mM glucose, or in other experiments, appropriate monosaccharide mixtures or phlorizin. Cholic acid was dialysed away overnight against the same buffer at *p*H 7.2. The sugar was labelled by allowing exchange with an appropriately labelled radioactive sugar in the medium for at least 30 min before each experiment. Initial experiments established the optimum ratio of phospholipid : cholic acid : brush border protein in the mixture before formation of the vesicles, as 60 : 30 : 1, a proportion similar to that used by Warren *et al.*[6]. Transport of sugars from the vesicles was measured by holding them within the cellulose hollow fibres of a Bio-Rad Bio-Fiber Mini-beaker. These fibres allow compounds of molecular weight < 5000 to diffuse freely into the fluid which was pumped through the beaker at a constant rate of 4.5 ml min^{-1}. Aliquots of perfusate were collected with a fraction collector and radioactivity measured by liquid scintillation spectrometry. The fluid used to perfuse the fibres was either of the same composition as the fluid against which the vesicles were dialysed (though containing neither glucose nor fructose) or a potassium–sucrose buffer containing identical amounts of equivalent potassium salts. Normally each perfusion involved an initial period during which sodium–sucrose buffer was perfused through the beaker and collected, and a second period of perfusion with potassium–sucrose buffer. Radioactive glucose or fructose released from the hollow fibres during the first period was from two sources—extravesicular fluid, and from inside the vesicles flowing down a concentration gradient. In the second period, amounts of monosaccharide from these first two sources were small, the majority being released from the vesicles when there is a sodium gradient from within the vesicles to the bathing fluid.

Figure 1 compares the release of glucose in vesicles containing brush border protein with those without protein. In Fig. 2 the outflow of glucose is seen in the absence of a sodium gradient together with the relative outflows of D- and L-glucose in the presence of a sodium gradient, D-glucose being transported more readily than its isomer. Total glucose release measured in tubes 40–60 inclusive was 3.11 μg D-glucose and 1.95 μg L-glucose (ratio D : L, 1.59). In a further experiment the release of D-glucose was 5.02 μg and L-glucose 4.07 μg (ratio 1.23). In both of these experiments phospholipid vesicles were loaded with a mixture of equal amounts of the two glucose isomers and the simultaneous release measured. A further experiment was performed in which half of a batch of vesicles was loaded with D-glucose, the other half with L-glucose;, here 10.66 μg and 6.67 μg L-glucose were released by the application of the sodium gradient (ratio 1.60).

Murer *et al.*[8] studied the uptake of the two glucose

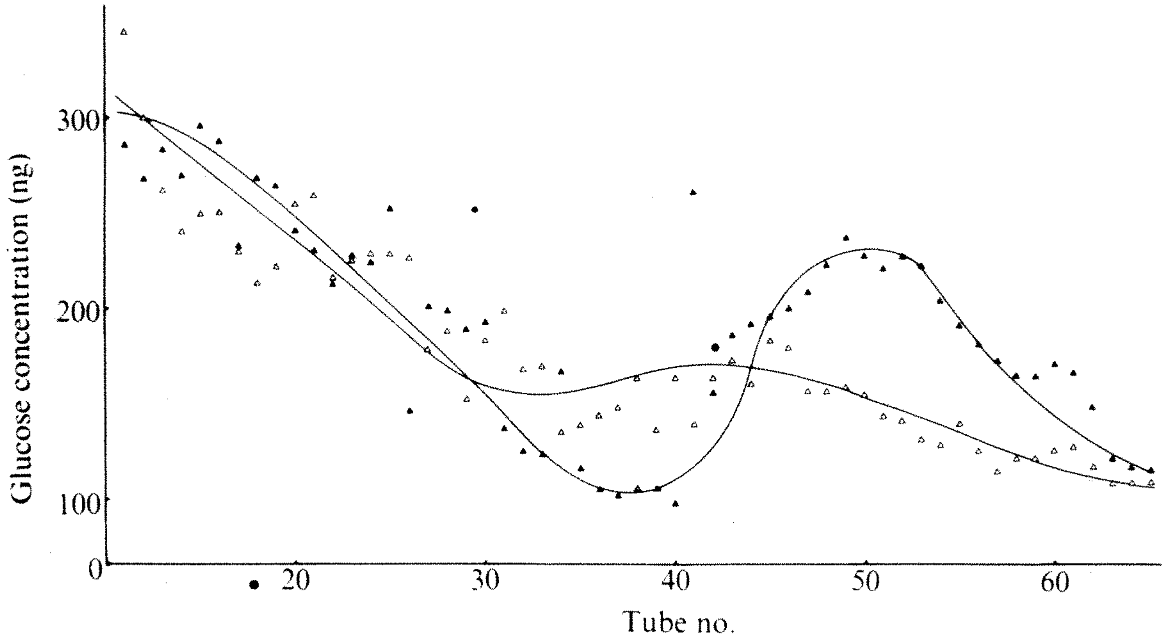
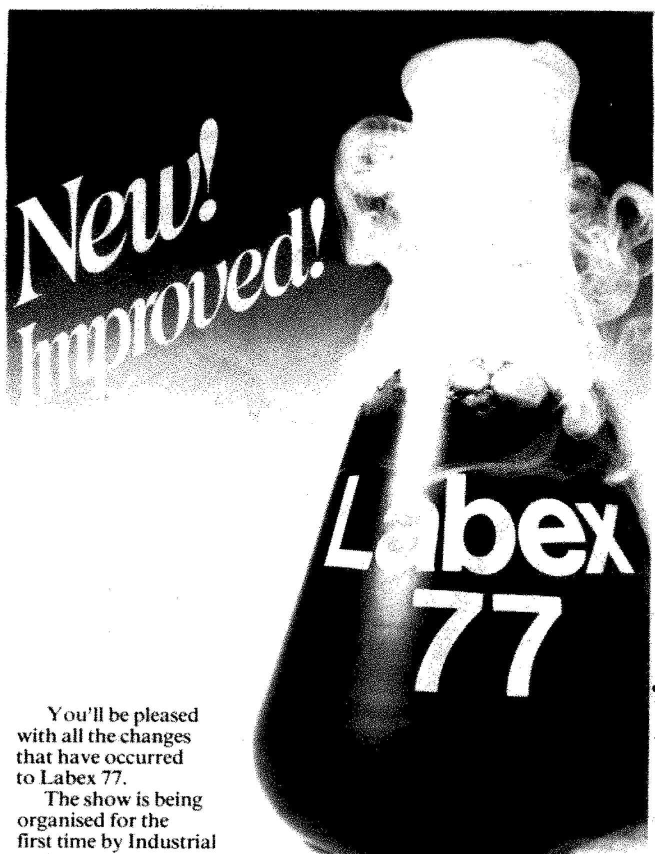


Fig. 1 Glucose release from phospholipid vesicles containing brush border protein (▲) and containing no protein ( ). The sodium gradient was introduced at tube 30 by perfusion with potassium–sucrose buffer. The delay in appearance of glucose from the vesicles containing protein is due to the deadspace in the system. Total glucose released from the protein-containing vesicles between tubes 40 and 60 was 4.03 μg, from those without protein 3.02 μg.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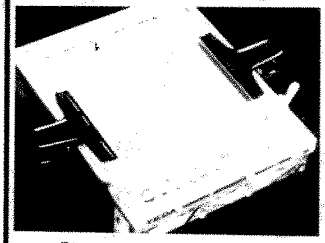

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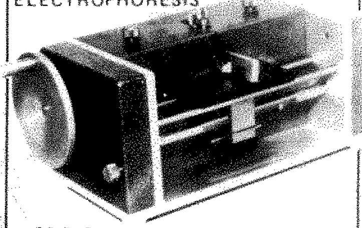

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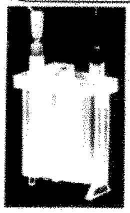

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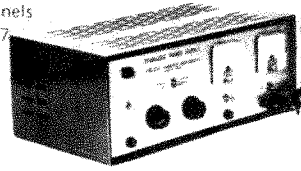

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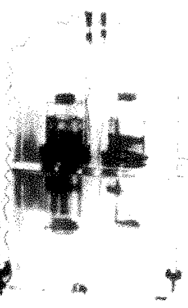

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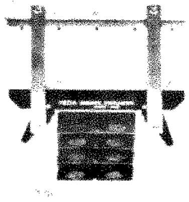

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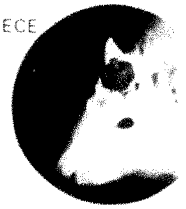

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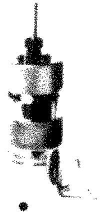

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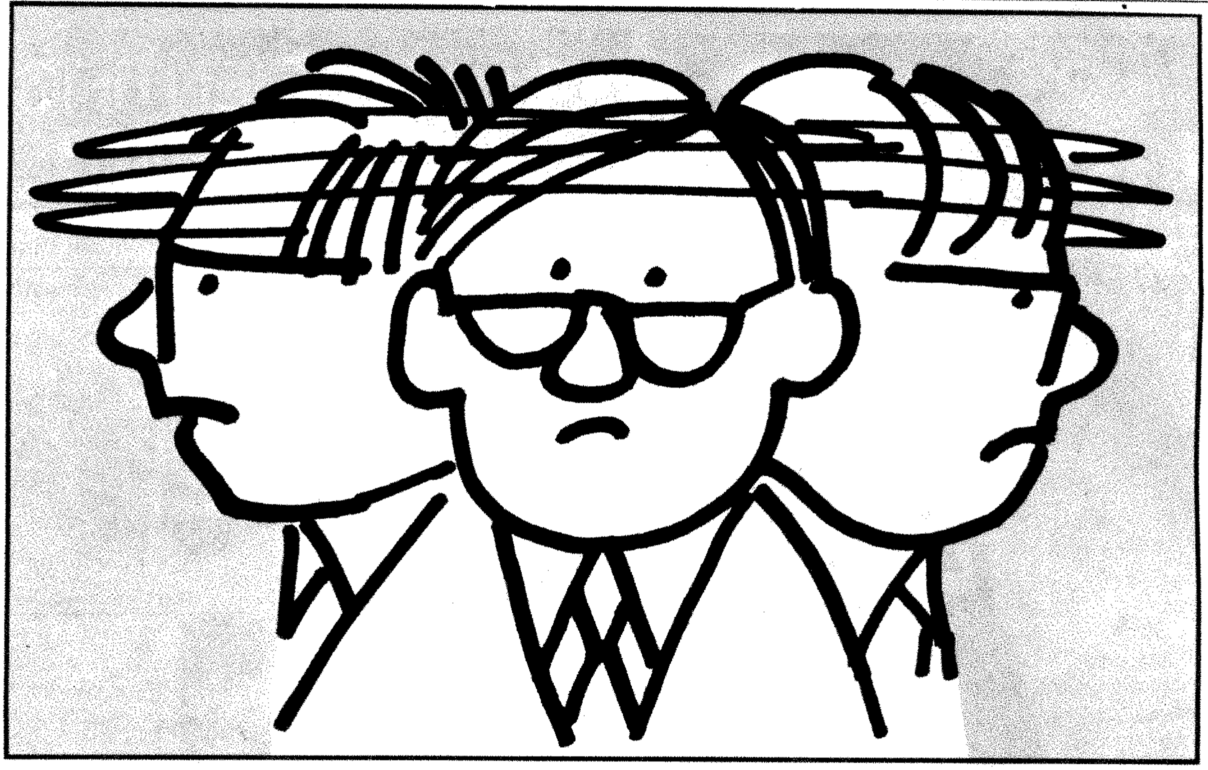

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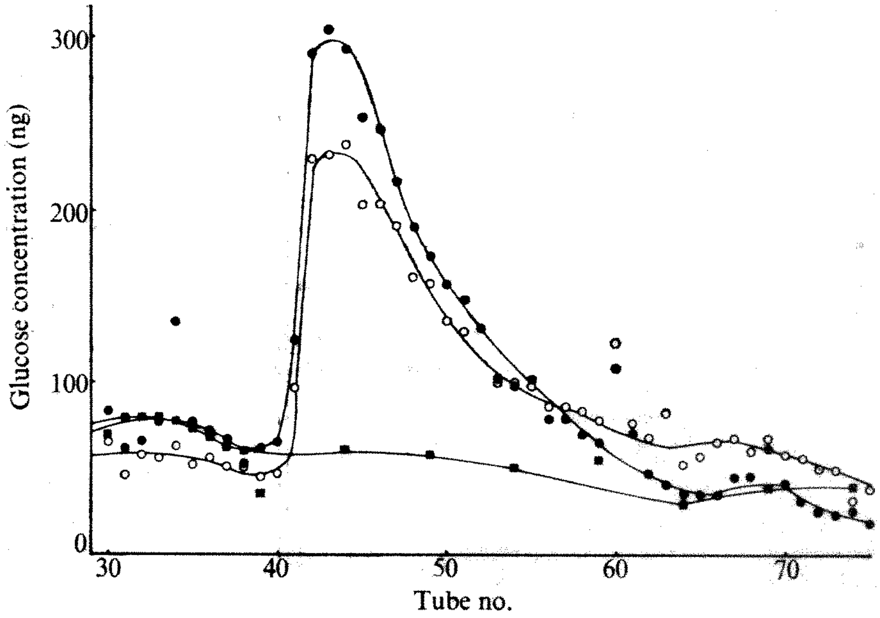
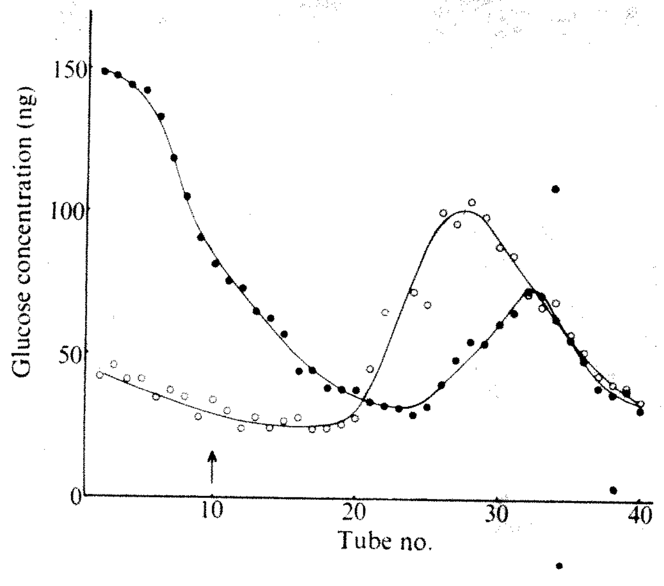
Fig. 2 Glucose release from phospholipid vesicles; *a*, in the absence of a sodium gradient (■); *b*, after loading with a mixture of D- and L-glucose, D-glucose being labelled with ^{3}H(●) and L-glucose with ^{14}C(○). The sodium gradient was introduced at tube 30.



isomers into isolated brush border vesicles using a filtration method; although after 120 s D-glucose uptake was 3.3 times greater than L-glucose, the ratio after 25 min was 1.4, similar to that seen in the present experiments.

Phlorizin (which inhibits transport of glucose both in small intestine and the renal proximal tubule) was incorporated into phospholipid vesicles at 5×10^{-4} M together with 2 mM glucose, and efflux of glucose was compared with controls containing no phlorizin (Fig. 3.) The initial loss of glucose from the vesicles down its chemical gradient was higher in the control than in the presence of phlorizin. After application of the sodium gradient, 0.84 μg of glucose was lost from the controls and 1.26 μg from the vesicles with phlorizin, the greater loss from the latter experiment probably being due to the larger amount of glucose which was still present in those vesicles. Total loss of glucose from control vesicles during the complete experiment was 2.7 μg, and in the presence of phlorizin, 1.9 μg. Fructose is not absorbed from the intestine by a sodium dependent mechanism, so the efflux of this sugar was compared with that of glucose. Vesicles were loaded with a mixture of ^{3}H-glucose 1.5 mM and ^{14}C-fructose 1.5 mM. Three perfusions were performed and the amounts of glucose released during the peak after the addition of the sodium gradient were 5.6, 5.1 and 5.14 μg, and of fructose, 2.11, 0.89 and 2.79 μg respectively. Furthermore, there was no significant rise in fructose efflux when the sodium gradient was introduced (Fig. 4).

Fig. 3 Release of D-glucose from phospholipid vesicles; *a*, control (●) and *b*, in presence of 5×10^{-4} M phlorizin (○). Vesicles were perfused with sodium sucrose buffer from samples 1–10. At the arrow it was changed to potassium–sucrose buffer. As in Figs 1 and 2 peaks show glucose release in response to the production of a Na$^+$ gradient.



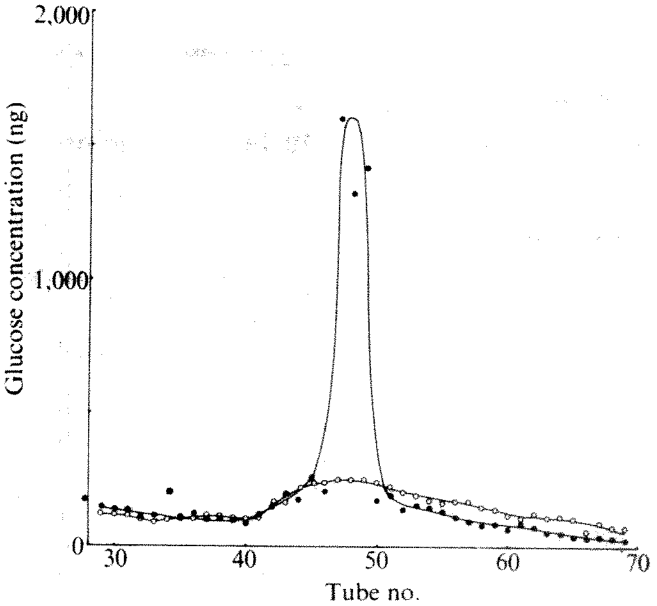


Fig. 4 Release of D-glucose (●) and fructose (○) from phospholipid vesicles in the presence of a sodium gradient introduced at tube 30.

The results of experiments using L-glucose and fructose might be explained if exchange of the labelled compounds with unlabelled sugar in the vesicles was not complete at 30 min. This is unlikely for three reasons. First, there was no change in the relative efflux of the two compounds when vesicles had been in contact with the isotope for varying times in excess of the initial 30 min. Second, the initial period, which consists at least in part of sugar from inside the vesicles, was very similar in D-glucose, L-glucose and fructose-loaded vesicles. Third, after the large sodium-dependent efflux of D-glucose the depleted vesicles released less than the vesicles containing a sugar, for example, L-glucose more resistant to release by a sodium gradient, so that in Fig. 1 the efflux of D-glucose crosses that of L-glucose after the peak efflux has occurred, and this was a consistent finding.

Our results show that a glucose transporting system can be reconstituted from phospholipid vesicles and protein from brush border membranes from small intestinal cell brush borders from pigs. This transport resembles the characteristics of transport of glucose in intact tissue in that it is increased in the presence of a sodium gradient, whereas the transport of a closely related sugar, fructose, is not. Furthermore, the reconstituted system distinguishes D-glucose from the L-isomer, and the total efflux of glucose is also reduced by phlorizin.

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Immunofluorescence shows localisation of prolactin to human amnion

AMNIOTIC fluid in man and rhesus monkey contains very high concentrations of prolactin[1–3], but its origins are unknown. It does not seem to come from the maternal circulation because isotopically labelled prolactin injected into the pregnant rhesus monkey does not appear in amniotic fluid[2]. One source of prolactin is suggested by the passage of foetal pituitary prolactin through the immature kidney into the urine and then into amniotic fluid[4,5]. But amniotic fluid and foetal prolactin differ in molecular size, suggesting different origins[4]. Incubated human amnion and chorion, but not placenta, release prolactin into the medium, indicating a second source of amniotic fluid prolactin[2]. Using indirect immunofluorescence, we report localisation of prolactin to the human amnion.

Placentae and amniotic membranes were collected from normal pregnancies of 12–40 weeks gestation, which had been terminated for psychiatric reasons in the second trimester by hysterotomy or had been delivered vaginally either prematurely or at term in the third trimester. The tissue was immediately cooled in ice and rinsed clean of blood and amniotic fluid with phosphate-buffered saline (*p*H 7.4). Within 90 min of delivery, tissue blocks (1 cm^3) of cotyledon, umbilical cord, placental and reflected parts of the amnion and the chorion laeve were snap frozen in isopentane liquid nitrogen at −70 °C. Specimens were also fixed in 10% phosphate-buffered formalin for conventional histology.

Frozen sections, 6 μm thick, were cut on a Lipshaw cryostat and examined by indirect immunofluorescence[6] using rabbit antisera to human prolactin obtained from the National Institutes of Health, Bethesda, Maryland. The reaction was traced with a fluorescein isothiocyanate-labelled goat anti-rabbit γ-globulin. The unabsorbed conjugate had a fluorescein to protein molar ratio of 4.8 : 1 and was absorbed with rat liver, kidney, gastrointestinal tract, sheep liver and human placenta so that it gave no nonspecific staining. The absorbed conjugate was used at a protein concentration of 8 mg ml^{-1}. After staining, the sections were examined by dark ground ultraviolet fluorescent microscopy using a condenser fitted with a colourless barrier filter and a toric lens beneath.

Immunological specificity of the reaction was assessed by reacting parallel control sections with conjugate alone, normal rabbit serum or rabbit anti-human prolactin neutralised by absorption with human prolactin, growth hormone or chorionic somatomammotrophin (placental lactogen). For neutralisation studies, 50 μl of rabbit anti-human prolactin was incubated overnight with 100 μl of human prolactin at a concentration of 6 μg ml^{-1}. The other hormones were used at similar concentrations. The rabbit anti-human prolactin serum was examined in a radioimmunoassay, and did not bind human growth hormone, insulin, chorionic somatomammotrophin or gonadotrophin, pituitary thyrotrophin and gonadotrophins or prostaglandins $F_2\alpha$ or E_1.

Multiple sections from seven pregnancies stained with rabbit anti-human prolactin showed specific immunofluorescence localised to the cytoplasm of the amnionic epithelium. Figure 1 demonstrates the uniform staining reaction throughout the cytoplasm of all epithelial cells. The intensity of immunofluorescence varied with the amnion of different patients and in general was more intense in the second trimester, but there seemed to be little variation in staining reaction between the placental and the reflected amnion from the same patient. In some sections, occasional blood vessels deep to the amnionic epithelium showed positive staining but this was never as intense as the staining of the overlying epithelium. No immunofluorescence was observed in the chorion laeve, umbilical cord or placental chorionic villi. Control section of amnion failed to stain when tested with conjugate alone or with normal rabbit serum. Antiserum absorbed with growth hormone or chorionic somatomammotrophin stained the amnionic epithelium but antiserum absorbed with human prolactin did not stain.

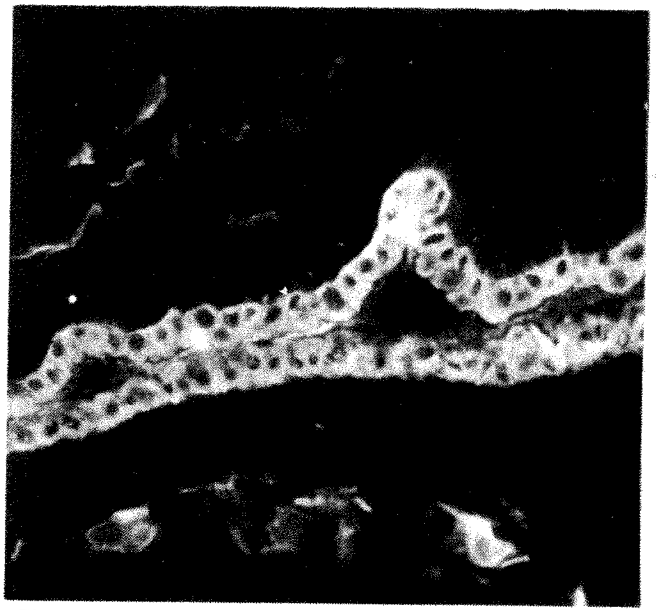

Fig. 1 Cytoplasmic immunofluorescence of a fold of human amnion epithelium from a 28-week pregnancy stained by rabbit anti-human prolactin. Small blood vessels are also stained (× 572).

We have demonstrated that prolactin is localised in the cytoplasm of human amnionic epithelium. It is possible that prolactin synthesis occurs in these cells, thus contributing to the relatively high concentrations of prolactin in amniotic fluid. The amnion epithelial cells are metabolically active[7] and contain rough endoplasmic reticulum capable of protein synthesis[8,9]. Although prolactin receptors have not been reported in human placental membranes or myometrium, this hormone influences water and electrolyte change in many species including man and may affect the volume and composition of human amniotic fluid[10–12]; in addition, it inhibits myometrial contraction[13]. Local production or concentration of prolactin by the epithelial cells of the amnion would facilitate these functions.

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Palatal cytosol cortisol-binding protein associated with cleft palate susceptibility and *H–2* genotype

GENETICALLY different inbred strains of mice have different susceptibilities to the production of cleft palate by a standard dose regimen of corticoids at the critical period during palatal organogenesis[1]. Because in target tissues corticoids form a specific cytoplasmic cortisol–receptor complex which interacts with the genome, resulting in activation or depression of transcription[2,3], we have postulated that susceptibility to corticoid-induced cleft palate is mediated by genetic differences in a cytosolic corticoid receptor protein(s) in palatal anlagen[4]. Other studies have suggested that susceptibility to cortisone-induced cleft palate is regulated by genes closely linked to the *H–2* locus[5]. We report here that the total level of cytosolic protein binders of ^{3}H-cortisol in maternal and foetal palates on day 11 of gestation correlates with susceptibility to cleft palate and with the *H–2* genotype. After separation of macromolecular bound hormone from free hormone on columns of Sephadex G-25 and further separation by microelectrofocusing[6], only one maternal and foetal palatal cytosol binder for ^{3}H-cortisol (pI 6.9–7.0) was found to correlate with susceptibility to cleft palate and the *H–2* genotype. Thus, a product of a gene in or near the *H–2* locus seems to be the glucocorticoid receptor, the level of which must be increased for a congenital malformation to occur.

The percentages of offspring in the different strains with cleft palate after maternal administration of corticoids were A/J, 100, DBA/1J, 92; C3H/HEJ, 68; C57BL/6J, 19 and CBA/J, 12, respectively[1]. This production of cleft palate by corticoids has been associated with the *H–2* genotype by the observation that the incidence of cortisone-induced cleft palate in C57BL/10 ScSn (B10) is 21%, and that of A/J is 100%, whereas that of B10.A, which has the genetic background of B10, but the *H–2*^{a} allele of A/J, is 81% (ref. 5). Foetuses of the sensitive A/J strain retain more ^{14}C-cortisone during the critical period than do those of the resistant strains, CBA/J (ref. 7) or C57BL/6J (ref. 8). Furthermore, synthetic corticoids are recovered in the unmetabolised state from foetal A/J tissues[10] and inhibit foetal RNA and protein synthesis at the critical period of cleft palate production[11]. A/J foetuses are more sensitive to an inhibition of RNA synthesis than those of the C3H/HEJ strain[12] and the effect is not due to a difference in metabolism of the steroid by A/J and C3H/HEJ strains[10].

A typical microelectrofocused pattern of ^{3}H-cortisol-binding proteins in the foetal jaws of each of the five inbred strains is shown in Fig. 1. Only one ^{3}H-cortisol-binding protein increased in amount in the different strains in proportion to the reported incidence of cleft palate produced by the standard cortisone regimen (indicated by arrows) (Fig. 1).

Most of the ^{3}H tightly bound to protein in the cytosol and nuclei of the foetal jaws was unmetabolised ^{3}H-cortisol (Fig. 2).

A linear regression analysis of each peak microelectrofocused showed a highly significant correlation between the amount of ^{3}H-cortisol-binding protein and the reported incidence of cleft palate in the seven strains: there was only one peak (pI 6.9–7.0) in both maternal palates ($r = 0.485$, $n = 27$; $P < 0.015$) and foetal jaws ($r = 0.585$, $n = 19$; $P < 0.016$) (Fig. 3).

A linear regression analysis also demonstrated that the total ^{3}H-cortisol-binding proteins in maternal ($r = 0.412$, $n = 30$, $P < 0.025$) and foetal ($r = 0.494$, $n = 18$, $P < 0.042$) palates of each of the seven strains was significantly correlated with the reported incidence of corticoid-induced cleft palate in that strain (data not shown). Moreover, when a pool of five adult male palates each of the B10 and B10.A strains taken 1 h after injection of 10 μCi ^{3}H-cortisol was analysed for this ^{3}H-cortisol-binding protein, the values plotted on the same linear regression curve as the maternal palates of the seven strains.

Our results show that the levels of foetal and maternal palatal cytosolic ^{3}H-cortisol-binding proteins (total and pI 6.9–7.0) are correlated with susceptibility to corticoid-induced cleft palate and are associated with alleles at the *H–2* locus. The *H–2* locus is not the sole determinant of ^{3}H-cortisol receptor levels, for C3H/HEJ (*H–2*^{k}) and CBA/J (*H–2*^{k}) have the same *H–2* genotype but different cleft palate susceptibilities and receptor levels. Our data suggest, however, that the ^{3}H-cortisol-binding protein with pI 6.9–7.0 is a candidate for the cortisol receptor in the mouse palate and that susceptibility to cortisol-induced cleft palate is mediated by this candidate for the cortisol receptor. A similar mechanism has been invoked to explain why medroxyprogesterone acetate produces cleft palate in

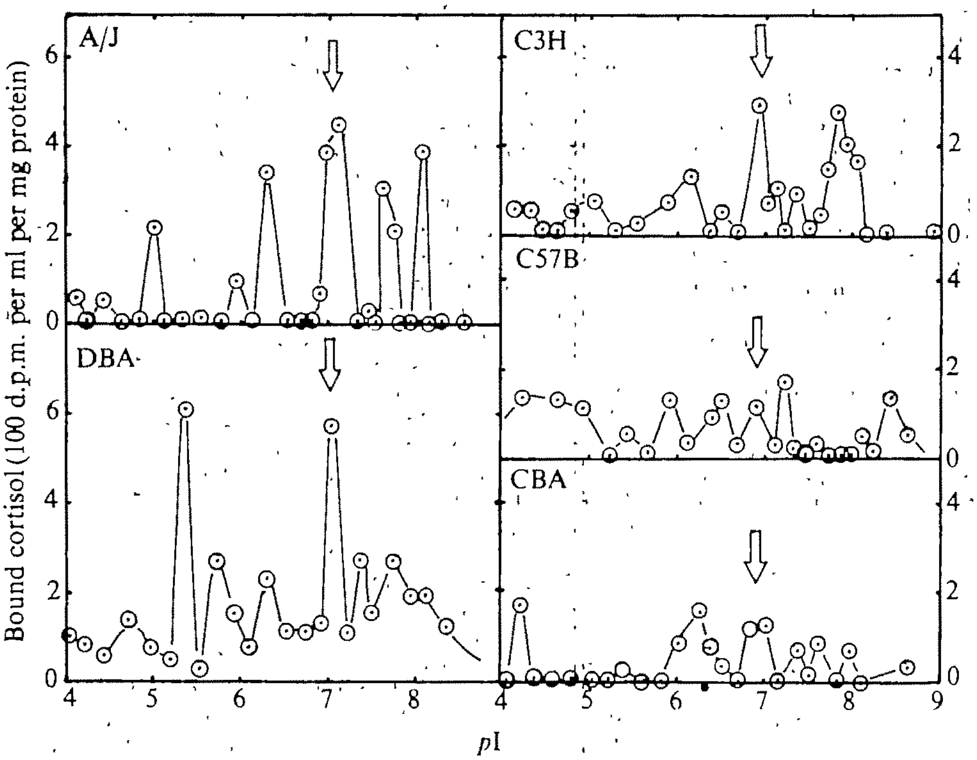

Fig. 1 Typical microelectrofocusing pattern of 1,2,6,7-^{3}H-cortisol-binding proteins in cytosol of foetal jaws after gel filtration through Sephadex G-25 Pregnant mice were killed by decapitation at 11 d of gestation (day of vaginal plug = day 0) 1 h after subcutaneous injection of 5 μCi of 1,2,6,7-^{3}H-cortisol (90 Ci mmol^{-1}). Maternal palates and the jaw regions (horizontal cut of the foetal head just below the level of the eyes including upper and lower jaws) of foetuses obtained by caesarian section were dissected, pooled (two or three maternal palates per pool and jaws of all foetuses in one or two litters per pool) and homogenised in a fivefold volume of 0.3 M sucrose–1 mM EDTA–20 mM Tris–50 mM phosphate buffer, pH 7.4 Cytosol was prepared, filtered through Sephadex G-25 and microelectrofocused as described before[6] with the exception that the columns were changed to 25-ml pipette and the Triton X-100 was eliminated. Each 0 5-ml sample was fractionated after electrofocusing at 200 V for 40 h. Radioactive samples were mixed with Formula 950A (New England Nuclear) and counted in a Packard Tricarb model 3375 scintillation spectrometer with a counting efficiency of 30–40% for ^{3}H Quenching was determined by an automatic external Standardisation ratio method. Protein was determined by the method of Lowry *et al.*[17]. The arrows indicate the *p*I 6 9–7.0 binding protein The amount of this protein is proportional to the reported incidence of cleft palate produced by four daily doses of 2.5 mg cortisone from days 11 to 14 of gestation in the different strains (Fig. 3). Each determination represents a pool of all the foetal jaws in one or two litters.

rabbits, but not in rats, since medroxyprogesterone acetate effectively competed with the binding ^{3}H-dexamethasone to the rabbit, but not to the rat liver glucocorticoid receptor[9].

We have found ^{3}H-cortisol largely in the unmetabolised state in the nuclei of the foetal jaws 1 h after maternal injection. This suggests nuclear uptake of a cortisol receptor, which presumably then results in repression or derepression of protein synthesis.

Detailed purification efforts reported so far by two groups have shown that the major rat[13,14], guinea pig[15] and human[16] liver glucocorticosteroid receptor capable of nuclear transfer *in vivo* and *in vitro* has an isoelectric point of either 7.1 and 6.1 after thermal activation[16], or 6.7 (refs 11–13). Other binding fractions found by us and the other groups do not seem to be receptors but transcortin (corticoid-binding globulin), ligandin (possibly an enzyme) or receptor fragments. The similarity of the *p*I of the ^{3}H-cortisol-binding protein associated with susceptibility to cleft palate with those of the liver glucocorticoid receptor found by the two groups makes the protein a candidate for a palatal glucocorticoid receptor mediating cleft palate.

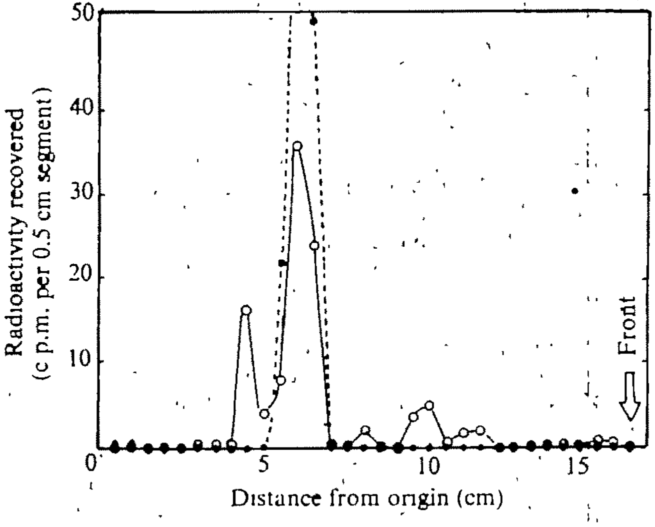

Fig. 2 From 75 to 80% of the ^{3}H in a 50% methylene chloride–chloroform extract of the cytosol and nuclei of the foetal jaws of the sensitive strains cochromatographed with an equivalent amount of ^{14}C-cortisol in a thin-layer chromatogram developed in cyclohexane–ethyl acetate–ethanol (45:45:10, v/v/v). The remainder of the ^{3}H was not identified accurately, but had the same mobility in this system as a non-radioactive cortisol metabolite, 3β, 11β-17α-, 21-tetrahydroxy-5α-pregnane. Circle and dot, ^{3}H in extract; ●, authentic ^{14}C-cortisol.

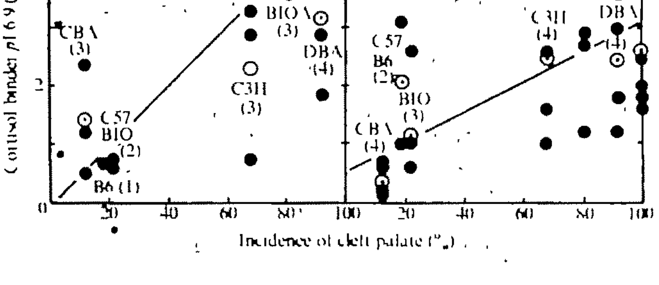

Fig. 3 Linear regression analysis of *p*I 6.9–7 0 receptor level of foetal jaws (*a*) and maternal palates (*b*) for each strain compared with reported mean incidence of cleft palate in each strain. The numbers in parentheses indicate the number of pools of adult palates or foetal jaws Circle and dot, mean value of all pools for each strain; ●, value of each pool.

The association between H–2 genotype and level of cortisol receptor in the cytosol suggests that the protein with pI 6.9–7.0 is either coded by a gene closely linked to the H–2 region or coded elsewhere in the genome, but is strongly affected by a gene in or near the H–2 region. Thus, the diversity of effects known to be associated with H–2 could be explained by a series of closely linked genes adapted to serve either immunological or hormonal functions.

After submission of our paper evidence in agreement with our findings was reported, to the effect that foetal A/J facial mesenchyme cells contain about twice the amount of saturable dexamethasone receptors than C57BL/6J mesenchyme cells obtained either directly or in primary culture[18].

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Neuroanatomical focus for morphine and enkaphalin-induced hypermotility

OPIATE receptors, through which all opiate alkaloids and peptides produce their pharmacological effects, are distributed heterogeneously in the mammalian brain[1–3]. The highest receptor densities seem to be associated with a number of limbic midbrain and forebrain structures that have been implicated in regulating motivation and emotionality[5,6]. Although the limbic midbrain region (periventricular-periaqueductal grey matter) is a critical focus for the analgesic[4–7] and depressant (A.P. and C.S., in preparation) effects of morphine and enkephalin[8], nothing is known about the functional significance of opiate receptors in the forebrain. Significantly, the nucleus accumbens (NA) in the limbic forebrain contains a relatively high concentration of opiate receptors[4] (C. B. Pert, personal communication). As this structure has a role in motility[9,10], an attempt has been made to ascertain whether it is an important focus for the excitatory effects[11,12] of opiates on behaviour. We report here that direct injections of morphine, D-Ala^2-enkephalin-amide (an enzyme-resistant enkephalin)[7,13] and apomorphine (a dopamine agonist) into the nucleus accumbens increased spontaneous motor activity in rats. The excitatory effects of morphine were antagonised by naloxone (an opiate antagonist) and not by haloperidol (a dopaminergic antagonist). Conversely, apomorphine was antagonised by haloperidol and not by naloxone.

Thirty-five male Sprague–Dawley rats, anaesthetised with sodium pentobarbital, were implanted stereotactically with bilateral intracerebral cannulae guides constructed from 23-gauge hypodermic tubing. The guide cannulae tips were aimed for a region 2 mm dorsal to the NA (AP 9.4, LAT 1.5, DV 2.0) (ref. 15). Drugs were injected into the NA through 30-gauge injectors which protruded 2 mm past the guide tips. Drug and control solutions were administered in a volume of 1 µl and at a rate of 1 µl per 30 s. One group of 10 animals was injected bilaterally in the NA with 5 µg morphine sulphate (that is, a 10 µg total dose), 10 µg apomorphine and 1 µl of water (the drug vehicle). All animals received both drugs and the vehicle in a counterbalanced order separated by 7 d. Another group of animals (n=9) was injected in the NA with both 10 µg D-Ala^2-enkephalin and 1 µl of water in a counterbalanced order, as above. Immediately after injections the animals were placed in Plexiglas chambers (23×23×15 cm) which were mounted in Motron motility meters (model 40Fc). Horizontal and vertical activity was monitored in 30-min segments for 6 h. Animals in these studies were always injected at 1000 h and removed from the apparatus at 1600 h. Six additional rats received injections of morphine and the vehicle into a region 2 mm dorsolateral to the NA. These rats were tested 30, 180 and 360 min after injection for 30 min.

Other groups of rats were injected with either 10 µg of apomorphine or 5 µg of morphine in the NA. Rats injected with apomorphine were pretreated with either naloxone (10 mg per kg body weight), haloperidol (0.25 mg per kg body weight) or normal saline administered intraperitoneally (i.p.) 15 min before being injected intracerebrally (i.c.). Rats injected with morphine i.c. were injected i.p. with either haloperidol, naloxone or normal saline 165 min later. Rats injected i.c. with morphine were placed in the apparatus 180 min after the i.c. injection. Rats injected with apomorphine were tested immediately after injection. These regimens were adopted to assure that both i.c. drug effects were at asymptotic levels during testing. All animals were tested for 30 min. Two groups of rats were also tested 15 min after i.p. injections of either naloxone and saline or apomorphine and saline. Rats in the above studies were tested between 0900 and 1500 h.

Apomorphine produced an immediate increase in both horizontal and vertical spontaneous activity after NA injections. This hyperactivity dissipated rapidly over 1 h and was no longer detectable 90 min after injection. Morphine, however, induced a more gradual increase in activity which reached asymptotic levels in approximately 3 h and was still present 5.5–6 h after injection. The excitatory effect of D-Ala^2-Met-enkephalin also had a gradual onset but was not as persistent as morphine (Fig 1).

Naloxone pretreatment antagonised the excitatory effects of morphine, whereas haloperidol was ineffective. Apomorphine, on the other hand, was antagonised by haloperidol and not by naloxone. Both naloxone and haloperidol decreased motor activity at the doses tested. Only the

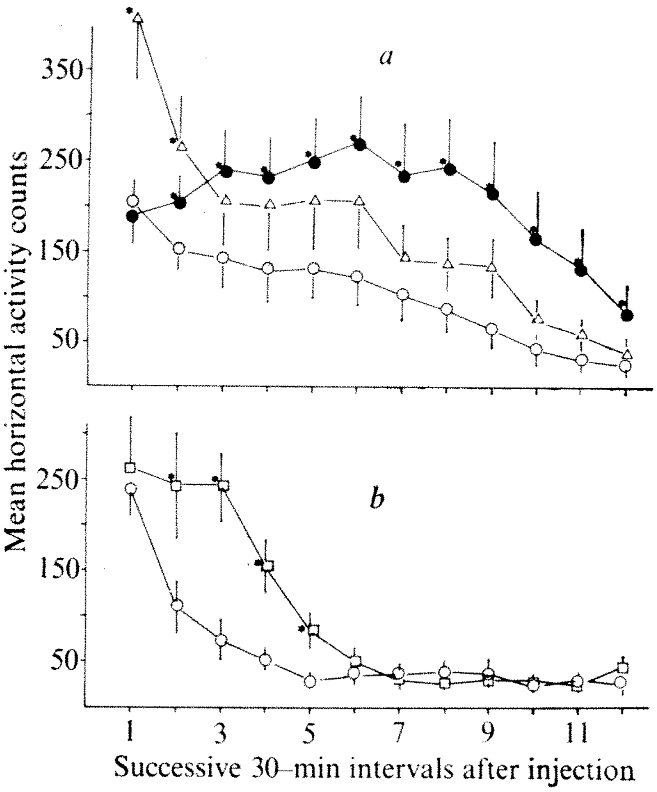

Fig. 1 Time course of horizontal hypermotility after bilateral injections of vehicle alone (○), morphine 5 µg (●), apomorphine 10 µg (▲) or D-Ala²-Metenkephalin 10 µg (□) into the NA. Repeated measures analyses of variance revealed a significant drug effect for both groups of rats (10 rats in *a*; 9 in *b*). Vertical lines indicate s.e.m. Similar results were obtained for vertical activity. * $P < 0.05$ for comparisons between effects produced by the drugs and the vehicle at each time point.

naloxone-induced hypomotility, however, differed significantly from the saline control level (Fig. 2). This finding seems to suggest the existence in brain of a tonically active enkephalinergic system with a role in modulating some aspects of motor activity.

Standard histological verification of injection sites at the termination of each study revealed that all injections had been made directly into the NA or into regions immediately bordering this structure. Injection sites in the anatomical control group, which had been implanted with cannulae aimed for structures outside the NA, were located in an area 1.5–2.5 mm dorsalateral, and in some cases anterior to, the NA. No hyperactivity was evident in this group after i.c. injections of morphine at any of the time points tested.

Both opiates[11,12] and various catecholaminergic agonists[15–17] have been reported to increase spontaneous motility in rodents after systemic injections. The NA, a terminal region for the mesolimbic dopamine pathways originating from area A10, has been implicated as a principal focus for the excitatory actions of amphetamine[9] and apomorphine[10,18]. These compounds affect activity either by increasing the availability of dopamine at the synapse, or by a direct action on dopamine receptors, as DA receptor blockers antagonise their excitatory actions. Opiates may also increase motility by activating the catecholamine systems[19–21]. Our findings, however, indicate that, at least in the rat forebrain, opiates produce excitatory effects that are dissociated from direct dopaminergic actions. An electrophysiological study, by Cayton and Bradley[22], of striatal neurones also seems to support the notion that dopamine and morphine (enkephalin) may exert identical effects on the same neurone or population of neurones through different receptor systems. These investigators reported that both morphine and dopamine inhibited the induced activation of neurones in the caudate nucleus after iontophoretic applications. The dopamine effect was reversed by a specific dopamine antagonist but not by naloxone while the morphine effect was antagonised by naloxone but not by the dopamine antagonist.

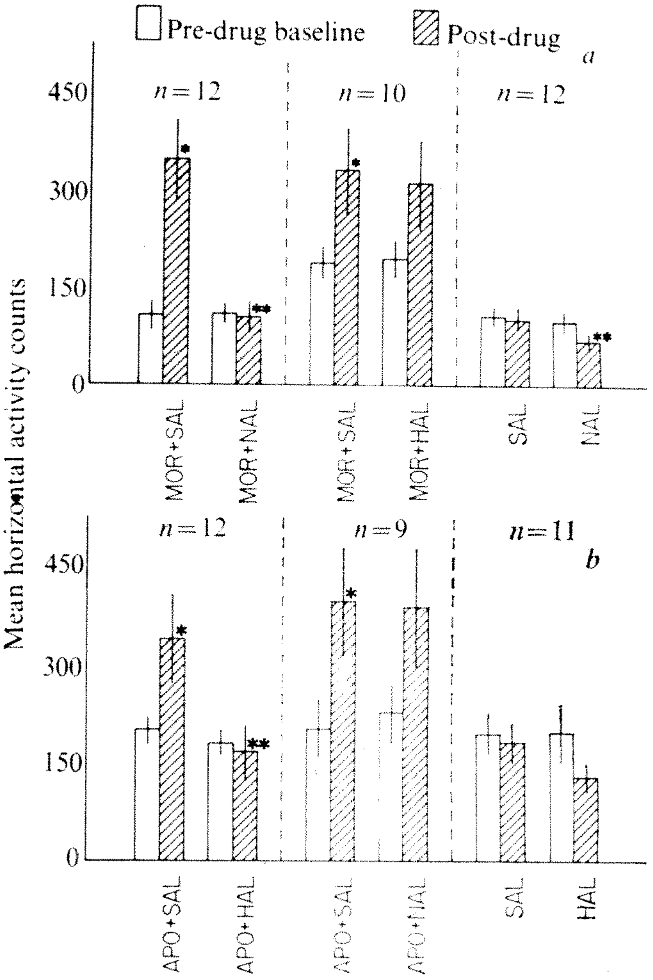

Fig. 2 Effects of naloxone (NAL) 10 mg per kg bodyweight i.p., haloperidol (HAL) 0.25 mg per kg bodyweight i.p. or saline (SAL) 1 ml per kg bodyweight pretreatment on horizontal hypermotility induced by injections of *a*, morphine (MOR), or *b*, apomorphine (APO) in the NA. Right side of figure illustrates the effect of systemically administered NAL (*a*) or HAL (*b*) on spontaneous motility. Similar results were obtained for vertical activity. Some animals were used for more than one drug interaction determination. All drug injections were spaced at least 5 d apart. Baseline activity measures were always determined 1 d before drug injection. Vertical lines indicate s.e.m. * $P < 0.05$ for comparison with baseline activities. ** $P < 0.05$ for comparison of MOR + NAL, and APO + HAL with MOR + SAL and APO + SAL, respectively.

It is possible that parallel neurohumoral systems exist in the forebrain to regulate motility—one encoded by dopamine and the other by enkephalin (the endogenous opiate neuromodulator or neurotransmitter)[8].

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Decreased foetal amino acid uptake, brain pyruvate kinase and intrauterine damage in maternal PKU

THE mammalian placenta can concentrate amino acids and release them into the foetal circulation, where they appear at levels higher than on the maternal side[1]. This mechanism provides a larger pool for foetal protein synthesis, but normal placental function can become a handicap in some situations. In the presence of an excess of amino acids the placenta exacerbates the maternal imbalance and produces a prolonged hyperaminoacidaemia in the foetus[2]. This in turn may affect the transplacental transport of other amino acids. Such can be the case in 'material phenylketonuria (PKU)', which often results in offspring with brain damage and mental retardation[3]. Using an animal model of 'maternal PKU' we have studied alterations in tissue levels and placental transport of L-tyrosine (Tyr), L-tryptophane (Trp) and certain foetal brain enzymes. We have found a reduced foetal tissue concentration of Trp and impaired foetal brain uptake of Tyr and Trp in the foetuses of hyperphenylalaninaemic dams, as well as reduced brain pyruvate kinase activity.

Pregnant rats from a homogeneous colony (CFN, Carworth) were fed either a commercial regular ration (C), or the same diet plus 7% L-phenylalanine (Phe) during the last week of gestation (HiPhe). Placental transport was investigated on the day before delivery. One hour before the procedure, HiPhe animals were given an oral bolus of Phe (500 mg kg^{-1}) to ensure consistent hyperphenylalaninaemia throughout the ensuing procedure, comparable with the situation of an untreated case of human PKU. In separate studies we determined that rats limited to the diet with 7% Phe would have 600–1,000 μM Phe in the blood. After anaesthesia with urethane (1.5 g kg^{-1}), rats were injected in the femoral vein with tracer amounts of metabolites tagged with tritium at 40 μCi kg^{-1}, as described[2] and applied[4] before. The identity of the label was checked by thin-layer chromatography and liquid scintillation counting. The brains of some foetuses in each litter were removed immediately and prepared for enzyme assays[5,6]. Free amino acid concentrations were determined in blood and tissues[7,8].

C foetuses had higher concentrations than the corresponding dams for all three aromatic amino acids tested. Foetuses of HiPhe rats had even higher blood Phe than the dams (Table 1). Blood Tyr concentrating were also higher in HiPhe animals. This is a well known feature of this animal model, because high Phe intake inhibits Phe hydroxylase only partially and does not prevent increased formation of Tyr from its precursor, Phe. If the rate of Tyr transport had been similar in both groups of rats, the concentrations on the foetal side in HiPhe foetuses would have exceeded the solubility of Tyr, approximately 3 mM in water at physiological temperatures. Although there were no differences in blood Trp levels in dams fed either one of the two regimes, the foetuses of hyperphenylalanineaemic mothers had very significantly lower blood and brain Trp.

Table 1 Maternal and foetal blood and brain amino acid concentrations, radioactivity after amino acid pulse

Group	Units	Phenylalanine Maternal blood	Phenylalanine Foetal blood	Phenylalanine Foetal brain	Tyrosine Maternal blood	Tyrosine Foetal blood	Tyrosine Foetal brain	Tryptophan Maternal blood	Tryptophan Foetal blood	Tryptophan Foetal brain
Controls	µM	165±41	315±67	355±48	65±11	365±26	798±102	30±6	106±2	126±13
HiPhe	µM	3,127±491‡	4,770±818	2,484±241‡	528±99‡	798±138*	936±62	34±4	48±9‡	68±6‡
Controls	d p m g^{-1}	—	—	—	66,438±7,159	51,694±4,337	44,581±1,320	38,278±3,390	50,883±4,131	40,735±4,392
HiPhe	d p m g^{-1}	—	—	—	66,027±6,295	53,972±3,964	37,335±2,222*	36,695±2,268	28,523±2,514‡	16,251±1,090†

Values represent the means ± s.e. for 7–18 dams and their litters. Specimens were taken 15 min after injection of the tracers. Foetal brains from whole litters were pooled for the assays. Either 3,5-^{3}H-L-tyrosine, 60 Ci mmol^{-1} or ^{3}H-L-tryptophan, 8 Ci mmol^{-1}, were given at a dose of 40 Ci kg^{-1}.
*$P < 0.02$ compared with controls.
†$P < 0.01$ compared with controls.
‡$P < 0.001$ compared with controls.

Fifteen minutes after pulse of ^{3}H-Tyr, the radioactivity present in maternal and foetal blood was not different in HiPhe and C animals. That reaching the brain, however, was lower in HiPhe foetuses. In contrast, after injection of ^{3}H-Trp, while counts in maternal blood were also similar in the two groups, foetal blood and brain radioactivity was significantly lower in the HiPhe group.

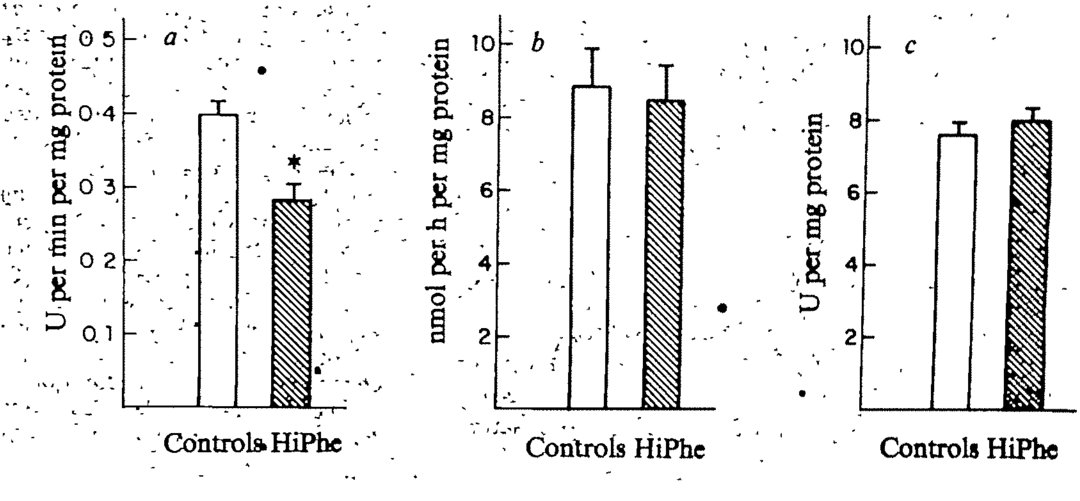

Fig. 1 Foetal rat brain enzymes from C and HiPhe dams. Pyruvate kinase (*a*) and hexokinase (*b*) were determined by the methods of Weber[5] and succinic dehydrogenase (*c*) by the procedure of Adlard and Dobbing[6]. Eight pooled litters were assayed in each group. T, means ±s.e.m. *, $P < 0.01$.

Among the brain enzymes studied, we found that pyruvate kinase was inhibited in HiPhe foetuses, while hexokinase and succinic dehydrogenase were not affected (Fig. 1). These results agree with the findings of several investigators[5,9,10] who showed *in vitro* and *in vivo* inhibition of rat and human brain pyruvate kinase by Phe. Their results yielded a K_i of of 3–5 mM on rat brain pyruvate kinase. Since the mean brain Phe concentration in the HiPhe foetuses was almost 2.5 mM (compare Table 1), seven times higher than in C, and of the same order of magnitude as the reported K_i the occurrence of this effect as a consequence of transplacental overflow of Phe was well justified.

Placental transport of Trp was investigated because the active transport of this amino acid may be inhibited competitively by Phe. It has been postulated that both share an active translocation mechanism[11]. The relevance of Trp to the problem of PKU is also tied to the defect in the metabolism of a Trp derivative, serotonin, considered to occur in that condition[12]. The synthesis of brain serontonin is regulated both by the availability of Trp and its hydroxylation by Trp hydroxylase, the rate-limiting enzymatic step[13,14]. In the animal model used here we have observed that the concentration of Trp in the brain of HiPhe foetuses is sharply decreased and that the transplacental passage of tritiated Trp is also lower in HiPhe dams, indicating that substrate availability is reduced in maternal hyperphenylalaninaemia. Moreover, it has been shown[15] that Phe at 10^{-4} to 10^{-5} M is inhibitory of Trp hydroxylase and Trp uptake by a brain stem synaptosome fraction. Thus, the findings on amino acid concentrations in foetal blood and brain suggest that foetuses exposed to the experimental conditions are at a disadvantage on two accounts for normal brain serotinin synthesis.

In previous studies we observed that hyperphenylalaninaemia was associated with an impaired transport across the small intestine of Phe and Trp, but not of Tyr[16]. A reduction of blood Phe levels was followed by a normalisation of intestinal absorption. In the present investigation we identified another biological membrane where the interaction between Phe and other aromatic amino acids can result in a severe impairment of the transport of Trp, and of Tyr, to a lesser extent. The resulting reduction in foetal blood and brain Trp, as well as the accumulation of Phe in foetal tissues, can provide a better understanding of the biochemical factors likely to be responsible for the *in utero* damage suffered by foetuses of hyperphenylalaninaemic mothers. Increasing foetal availability of Trp by dietary manipulations[17] may have a possible impact of equal magnitude as decreasing maternal blood Phe, a therapeutic approach already suggested in the prevention of otherwise irreversible damage occurring in human 'maternal PKU'[13,18].

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Physiological significance of α-adrenoceptor-mediated negative feedback mechanism regulating noradrenaline release during nerve stimulation

NORADRENERGIC nerve endings in the peripheral nervous system are involved in the synthesis, storage, release and uptake of the neurotransmitter. The view that released noradrenaline interacts only with the postsynaptic receptors of the effector cell to elicit the typical response (contraction of a smooth muscle, positive chronotropic and inotropic effects) has been challenged by the proposal that specific receptors might also be present in the outer surface of the membrane of nerve endings[1–5]. These presynaptic receptors are of the α-adrenergic type, mediating a negative feedback mechanism regulating noradrenaline release during nerve stimulation[1–5]. The noradrenaline thus released, on reaching a threshold concentration in the synaptic gap, activates presynaptic α adrenoceptors triggering a negative feedback mechanism that inhibits further release of the transmitter. In support of this view, it has been reported that activation of presynaptic α adrenoceptors leads to a decrease in transmitter release, while blockade of these receptors results in an increase in noradrenaline release during nerve stimulation[5]. We have now demonstrated that the negative feedback mechanism mediated by presynaptic α adrenoceptors has a physiological role in neurotransmission.

In tissues where the effector organ's responses are mediated by α adrenoceptors, block of the presynaptic α receptors enhances noradrenaline release by nerve stimulation, but decreases the responses by blockade of the postsynaptic receptors[2,6]. On the other hand, for tissues in which the responses are mediated by postsynaptic β adrenoceptors, block of presynaptic α receptors should increase noradrenaline release and the responses to sympathetic nerve stimulation. Nevertheless, in the perfused cat heart[7] and in isolated guinea pig atria[8] there are no significant increases in the positive chronotropic responses to nerve stimulation in conditions in which the release of noradrenaline was increased by phentolamine.

An enhancement in transmitter release which is not accompanied by an increase in the response of the effector organ might indicate that the negative feedback mechanism mediated by presynaptic α adrenoceptors does not play a physiological role in noradrenergic neurotransmission. To explore this possibility further, the responses to accelerans nerve stimulation and the increase in transmitter release in isolated guinea pig atria were investigated during and after exposure to the α-receptor blocking agent, phentolamine.

Guinea pig atria with their accelerans nerve, in an isolated organ bath fitted with platinum electrodes for nerve stimulation[9], were immersed in Locke's solution with 1.4 μM atropine. The spontaneous contractions of the preparation were recorded with a force displacement transducer connected to a Grass polygraph. The endogenous noradrenaline stores were labelled with $(\pm)$-7-^{3}H-noradrenaline[9] and the accelerans nerves were stimulated with square pulses of 0.5 ms duration and supramaximal voltage.

The overflow of labelled transmitter elicited by nerve stimulation was calculated as fractional release per shock :

Table 1 Effects of phentolamine on ^{3}H-noradrenaline release elicited by nerve stimulation in guinea pig atria

		Fractional release per shock ($\times 10^{-4}$)			Ratio	
Experimental group	*n*	S_1	S_2	S_3	S_2/S_1	S_3/S_1
Control	6	1.11 ± 0.18	1.12 ± 0 22	0.89 ± 0 17	1 03 ± 0 11	0 80 ± 0.09
Phentolamine 0.31 μM	6	1.38 ± 0 28	4.42 ± 0.55†	2.33 ± 0 30*	3 65 ± 0.62*	1.92 ± 0 30*
Phentolamine 3.1 μM	6	1.23 ± 0.14	6.67 ± 1 65†	4.39 ± 0 76†	6.09 ± 0.73‡	3 51 ± 0 30‡

Fractional release per shock is expressed as total nCi released per shock divided by the total nCi remaining in the tissue at the onset of nerve stimulation (4 Hz, during 60 s, with supramaximal voltage). Phentolamine (0.31 or 3.1 μM) was added to the medium 30 min before S_2 and washed out 5 min after S_2. The interval between each period of nerve stimulation was 50 min. Mean values ± s.e.m. are shown *n*, Number of experiments.

* $P < 0\ 05$ compared with the control.

† $P < 0.025$ compared with control.

‡ $P < 0\ 005$ compared with control

total nCi released per shock divided by the total nCi remaining in the tissue at the onset of stimulation[10] Dose–response curves to exogenous noradrenaline were determined cumulatively[11].

During exposure to 0 31 μM phentolamine there was a nearly fourfold increase in ^{3}H-transmitter release elicited by nerve stimulation (Table 1). The enhancement in transmitter release was still observed after the α-blocking agent was removed from the perfusion medium (Table 1)

We studied, separately, the positive chronotropic effects of accelerans nerve stimulation at low frequencies After four control periods of nerve stimulation, 0 31 μM phentolamine was added to the medium and a significant increase in the responses to nerve stimulation was obtained (Fig. 1) As observed for the effects of phentolamine on ^{3}H-transmitter release, the potentiation of the chronotropic responses elicited by nerve stimulation persisted after the α-receptor blocking agent was removed from the perfusing medium (Fig 1)

The effects of phentolamine on transmitter release were concentration-dependent (Table 1) When the positive chronotropic responses to nerve stimulation were determined in the presence of 3.1 μM phentolamine, however, the increase in responses to nerve stimulation was not statistically significant in the controls the rate was increased by 31 ± 7 beats per min, while in the presence of 3 1 μM phentolamine the increase was 47 ± 8 beats per min ($n = 10$, $P > 0.05$) The absence of a significant potentiation in the responses to nerve stimulation in the presence of 3 1 μM phentolamine seems to be related to the ability of the α-receptor blocking agent to elicit a negative chronotropic effect and to antagonise responses of the atrial pacemaker to noradrenaline. Exposure to phentolamine produced a negative chronotropic effect which was concentration-dependent The maximum decrease in atrial rate was obtained with 31 μM phentolamine a reduction by 51 ± 4 beats per min (mean ± s e m of 13 experiments) During exposure to 3 1 μM phentolamine the rate decreased by 16 ± 3 beats per min ($n = 13$) The negative chronotropic effect of phentolamine seems to be veratramine-like[12] since there was a significant correlation between the initial rate and the magnitude of the negative chronotropic effect of phentolamine: the higher the atrial rate, the more pronounced the negative chronotropic effect ($r = 0\ 708$, $P < 0\ 01$, $n = 25$).

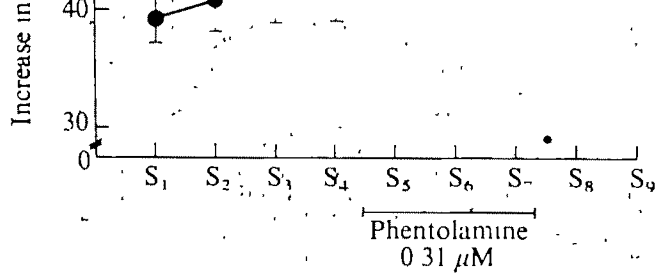

Fig. 1 Potentiation by phentolamine of the positive chronotropic effects of accelerans nerve stimulation in isolated guinea pig atria Increase in atrial rate induced by nerve stimulations S_1–S_9 at 0.5 Hz, for 10 s, supramaximal voltage The interval between each nerve stimulation was 20 min Phentolamine (0 31 μM) was added to the medium 10 min before S_4 and kept until 10 min after S_7 In the absence of phentolamine the responses to nerve stimulation were sustained from S_1 to S_9 Mean values ± s.e m of 11 experiments are shown * $P < 0.05$ compared with S_1.

Exposure to 31 μM phentolamine produced a marked negative chronotropic effect and a shift to the right in the dose–response curve to exogenous noradrenaline (Table 2).

Table 2 Antagonism by phentolamine of the positive chronotropic responses to noradrenaline in isolated guinea pig atria

			Responses to (−)-noradrenaline	
Experimental group	*n*	Initial rate* (beats per min)	pD_2†	Maximum increase‡
Controls	6	209 ± 9	6.82 ± 0.13	119 ± 10
Phentolamine 31 μM	8	136 ± 8‖	5.54 ± 0.27§	135 ± 7

* Rate before the beginning of the concentration effect curve to exogenous noradrenaline

† Mean negative log molar ED_{50}.

‡ Maximum increase in atrial rate (in beats min^{-1}) in response to (−)-noradrenaline

Mean values ± s.e m are shown *n*, number of experiments

§ $P < 0\ 05$ compared with control.

‖ $P < 0\ 001$ compared with control.

Phentolamine (3.1 μM) also reduced the positive chronotropic responses to noradrenaline. The positive chronotropic effects of 6 and 18 nM noradrenaline were 15 ± 2 and 22 ± 4 beats per min, respectively (mean ± s.e m. of six observations). In the presence of 3.1 μM phentolamine these values were reduced to 4 ± 1 and 10 ± 3 beats per min (mean ± s.e.m. of seven observations, $P < 0\ 025$ and $P < 0.001$, respectively).

The antagonism by phentolamine of responses of the atrial pacemaker to noradrenaline probably explains why the responses to accelerans nerve stimulation were not increased, during exposure to 3.1 μM α-receptor blocking agent, in spite of the considerable increase in transmitter release (Table 1).

With the concentration of phentolamine lacking negative chronotropic effects (0.31 μM), a significant increase in responses to nerve stimulation was obtained (Fig. 1) when the release of noradrenaline was enhanced (Table 1). This increase in response supports the view that the negative feedback mechanism mediated by presynaptic α-adrenoceptors plays a major physiological role in the regulation of noradrenergic neurotransmission.

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Rapid test to detect agents that damage human DNA

PERHAPS the most successful screening test to detect potential mutagens and/or carcinogens is that of Ames *et al*[1], using *Salmonella*. Attempts to use mammalian cells have generally involved measuring some cellular response to DNA damage, for example, induction of DNA repair[2]; if the response implies that an agent has damaged the DNA, the agent is considered to be a potential mutagen and/or carcinogen. An early response to DNA damage in human cells is inhibition of DNA synthesis. But certain non-DNA-damaging agents also inhibit DNA synthesis. I show here that DNA-damaging and non-DNA-damaging agents can be distinguished by the changes in the rates of DNA synthesis after treatment. with a DNA-damaging agent, the rate of DNA synthesis continues to decrease, but with a non-DNA-damaging agent the rate of DNA synthesis increases after treatment.

The test measures thymidine uptake into the DNA of human (HeLa) cells at various times after treatment with a presumptive mutagen or carcinogen The results with several known mutagenic agents (Fig. 1*a*–*d*) show that in every case the amount of thymidine incorporated into the DNA decreased during the first 1–2 h after the end of treatment (after removal of the medium containing the chemical or removal of the cells from the radiation field). Ethyl methanesulphonate (EMS) gave results similar to those with methyl methanesulphonate (MMS), except that 10 mM EMS was required to obtain positive results (data not shown). Benzpyrene requires metabolic activation, and when benzpyrene was incubated with HeLa cells and a microsomal preparation from rat liver, the S-9 mixture[1], it was active, whereas alone it was not. In some experiments ^{3}H-leucine was used to measure protein synthesis during the same time that ^{14}C-thymidine was used to measure DNA synthesis. This is illustrated for 4-nitroquinoline-1-oxide (4NQO) only, but in all cases tested the effect of the agent on protein synthesis was less than the effect on DNA synthesis.

Agents that inhibit DNA synthesis but do not damage DNA—cycloheximide, hydroxyurea, dimethyl sulphoxide, and $NiCl_2$—were also tested (Fig. 2) In contrast to the results with DNA-damaging agents, the rate of thymidine incorporation into DNA increased rapidly immediately after the removal of these agents from the medium (although complete recovery sometimes took a few hours). Phorbol myristate acetate, a so-called promoter of carcinogenesis, was also found in this class (data not shown).

We explain this difference between the two classes of agents that inhibit DNA synthesis as follows When an agent that inhibits by metabolic means is removed from the medium, the metabolic block is removed, recovery begins immediately, and the rate of DNA synthesis increases. When a DNA-damaging agent is removed from the medium,

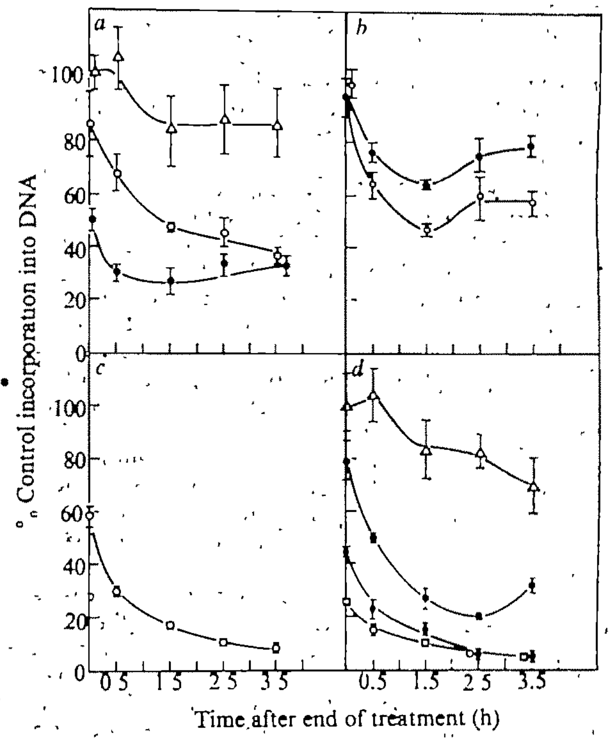

Fig. 1 Rate of DNA synthesis, measured as percentage of control incorporation of thymidine into DNA, in HeLa cells at various times after treatment with *a*, benzpyrene; *b*, MMS; *c*, ultraviolet light (10 J $\mathrm{m^{-2}}$), *d*, 4NQO *a*, △, 7 μM; ○, 7 μm + S-9; ● 20 μm + S-9. *b*, ●, 0.5 mM, ○, 1 mM. *d*, △, 0.2 μM (protein synthesis); ●, 0.2 μM; ◆, 1 μM; ○, 2 μM HeLa cells were grown in Eagle's minimal essential medium (MEM) with 15% calf serum 4NQO was dissolved in alcohol at 0.2 mM Benzpyrene was dissolved in alcohol at 1 mM and diluted into McCoy's 5a medium containing 2.5% calf serum and a 1/20 dilution of S-9 mix, a microsomal preparation from rat liver prepared from animals previously injected with Aroclor, a polychlorinated biphenyl[1] Some cultures were treated with benzpyrene in McCoy's medium without S-9 mix The S-9 mix was simultaneously tested and found to have no effect on DNA or protein synthesis at the dilutions used Incubation times were 30 min for MMS and 4NQO and 1 h for benzpyrene Ultraviolet light irradiations were performed on cells under saline at 1.3 J $\mathrm{m^{-2}\,s^{-1}}$. Immediately after incubation with the chemical or after exposure to ultraviolet light, the medium was removed from all cultures (including controls, which were treated identically except for treatment with the agent to be tested), and Eagle's MEM was placed back on all but two controls and two cultures treated with the agent These were immediately incubated for 10 min with MEM containing either ^{14}C-thymidine (0.3 μCi $\mathrm{ml^{-1}}$, 50 mCi $\mathrm{mmol^{-1}}$) and ^{3}H-leucine (10 μCi $\mathrm{ml^{-1}}$; 84 Ci $\mathrm{mmol^{-1}}$) or ^{3}H-thymidine (10 μCi $\mathrm{ml^{-1}}$; 50 Ci $\mathrm{mmol^{-1}}$) alone. (When ^{3}H-thymidine was used, the cells had been incubated for 24 h or more with 0.02 μCi $\mathrm{ml^{-1}}$ ^{14}C-thymidine before treatment with the agent.) At each of the times indicated, two more controls and two more treated cultures were incubated in radioactive medium for 10 min. The cells were washed after each incubation, scraped into SSC (0.15 M sodium chloride, 0.015 M sodium citrate), and filtered through GF/C (Whatman) glass filters moistened with cold 4% perchloric acid The filters were washed with cold 4% perchloric acid, 70% alcohol, and 100% alcohol, dried, and added to vials containing scintillation fluid, their ^{14}C and ^{3}H content was measured by liquid scintillation spectrometry. Error bars indicate the range of values obtained in a single experiment.

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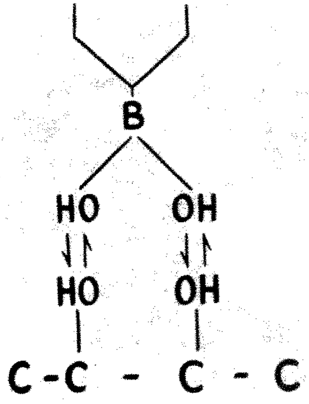

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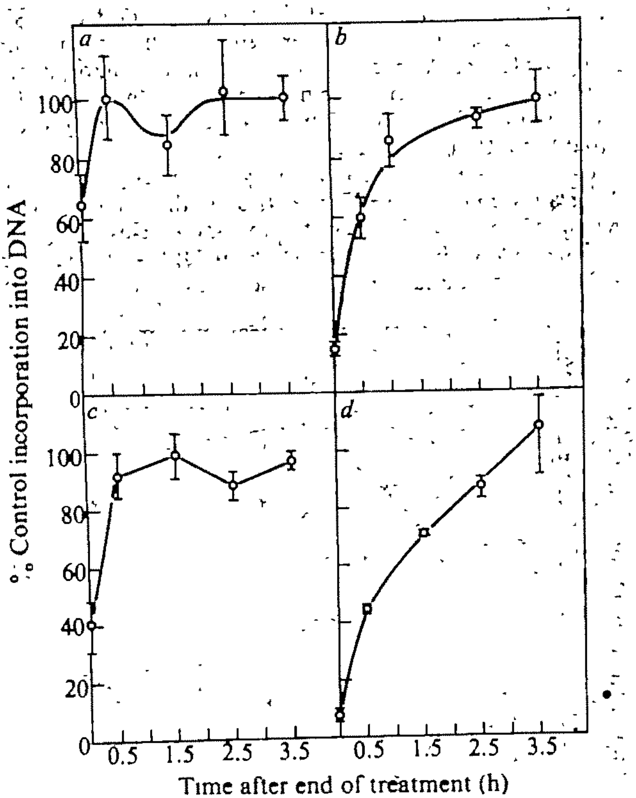

Fig. 2 Rate of DNA synthesis in HeLa cells at various times after treatment with agents that inhibit DNA synthesis by metabolic mechanisms. Conditions were the same as for Fig. 1. *a*, $NiCl_2$ (5 mM); *b*, DMSO (1.3 M); *c*, cycloheximide (23 μM); *d*, hydroxyurea (1 mM).

however, the damage remains, and the rate of DNA synthesis decreases with time. This occurs for one or both of two reasons. First, certain agents, such as ultraviolet light, induce lesions that block the progression of growing points in replicating DNA[4,5] so that progressively fewer growing points remain in operation, until repair occurs. Second, other agents, such as X rays[6–8] and MMS[9], block initiation of replicons, so that as more and more replicons terminate after damage is incurred, DNA synthesis progressively decreases[10].

My results suggest that measurements of DNA synthesis within the first 1–2 h after treatment with an agent can determine its potential as a DNA-damaging agent and therefore as a mutagenic and potentially carcinogenic agent. The simplest procedure is to label HeLa cells for one generation or more (≥24 h) with ^{14}C-thymidine before treatment and to pulse with ^{3}H-thymidine for 10 min at various times after treatment, comparing the resulting $^{3}H/^{14}C$ ratios with those measured at the same times in controls. Although, in many of the experiments reported here prelabel was omitted, so that synthesis of both protein (with ^{3}H-leucine) and DNA (with ^{14}C-thymidine) could be measured after treatment, it is not necessary to measure the former to detect DNA-damaging agents. Furthermore, the use of ^{14}C-thymidine to label parental DNA before treatment with the suspected agent makes possible greater flexibility; for example, care need not be taken to put exactly the same number of cells in each plate, because the resulting $^{3}H/^{14}C$ ratios are independent of cell number.

The experiment using S-9 demonstrates that the method can detect agents requiring metabolic activation. Cyclophosphamide, which also requires activation, required a 2.5× greater concentration of S-9 to achieve activation (data not shown) than did benzpyrene. Thus, each agent needs to be tested for the optimal conditions for activation, as has been reported for other detection systems[3].

The results with $NiCl_2$ require comment. Nickel in the metallic form has been shown to be carcinogenic in rodents[11]. Unpublished work in my laboratory, however, has shown that $NiCl_2$ inhibits DNA synthesis by slowing (not blocking) the rate of chain elongation, in a manner similar to cycloheximide[12] and hydroxyurea[13], and therefore is presumably not a DNA-damaging agent.

The advantages of my method for detecting DNA-damaging agents are: (1) HeLa cells are of human origin and are probably representative of the reaction of other human cells—presumably other rapidly growing human cells could also be used; (2) HeLa cells are easy to grow and do not exhibit contact inhibition, so that enough cells can easily be obtained in a 35-mm Petri dish or other small test vessel for a single determination; (3) the test requires only about 2 h from the first addition of the agent to the end of the last incubation, and (4) the assay is extremely simple—no extraction procedures are necessary because the whole cells are precipitated on to filters, which are dried and counted by conventional procedures. The disadvantages are similar to those of other systems based on DNA damage, for example the system will not detect mutagens, such as asbestos, that act through unknown mechanisms. False positives do not seem to be a factor. Possibly some agent could act so that metabolism in general, and consequently DNA synthesis, would continue to decrease with time after the agent has been removed from the cell. Experiments with KCN, however, suggest that this is unlikely. KCN at 15 and 30 mM decreased the DNA synthesis rates to about 25 and 15% of the controls, respectively; nevertheless, after KCN was removed, recovery of DNA synthesis began immediately for the lower concentration and within 1.5 h for the higher. Thus, recovery of DNA synthesis occurs from even a powerful general poison, as long as no DNA damage is incurred. It seems possible, therefore, that this simple test might be useful as a screening procedure to detect agents that damage human DNA.

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Functional identity of a mouse ascites and a rabbit reticulocyte initiation factor required for natural mRNA translation

INITIATION of eukaryotic protein synthesis involves the assembly of a functional initiation complex from components including ribosomal subunits, initiator tRNA (Met-$tRNA_F$), initiation factors, messenger RNA, ATP and GTP[1–3]. Much research in this area has centred on the possible control of eukaryotic translation at the level of polypeptide initiation, as this step seems to be rate-limiting in the overall synthetic reaction[4,5]. Some studies have suggested the existence of tissue-specific initiation factors[6], others have described initiation factors which apparently discriminate between eukaryotic mRNAs[7]. Work in this laboratory led to the purification of an initiation factor of molecular weight 53,000 from the supernatant fraction of Krebs II ascites cells[8]. This factor ($\mathrm{IF_{EMC}}$) was shown to be required for the translation of encephalomyocarditis virus RNA (EMC RNA) but not for globin mRNA translation in a fractionated ascites cell-free system[8]. Other studies on the translation of mRNAs injected into living *Xenopus laevis* oocytes[10], and on the translation of various mRNAs in several crude eukaryotic protein-synthesising systems[11] have suggested, however, that eukaryotic translation proceeds without controls exerted by specific initiation factors. We have adopted a comparative approach to this problem, examining the translation of various eukaryotic mRNAs in a fractionated cell-free system from Krebs II ascites cells, and testing the specificity of two mammalian factors reported to be required for the initiation of natural mRNA translation.

The specificity of the $\mathrm{IF_{EMC}}$ factor was investigated further (Table 1). Our results confirm work demonstrating requirement for $\mathrm{IF_{EMC}}$ in EMC RNA translation[8]. Translation of mengo virus RNA and foot-and-mouth disease virus RNA was also dependent on the addition of $\mathrm{IF_{EMC}}$. Some mRNAs, however, showed no significant dependence on $\mathrm{IF_{EMC}}$ for translation (for example, tobacco mosaic virus RNA), whereas others showed a partial stimulation of translation by $\mathrm{IF_{EMC}}$ (for example, Semliki forest virus 26S RNA). Our studies have shown a wide spectrum of dependencies on $\mathrm{IF_{EMC}}$ for the translation of several mRNAs, and have demonstrated differences in the efficiency of their translation in the fractionated cell-free system. For example, RNAs from two closely related picornaviruses[14], namely EMC and polio, show differences both in their extent of translation and in their requirement for $\mathrm{IF_{EMC}}$. It is therefore possible that other factors, necessary for translation of mRNAs such as polio RNA, may be either absent or present in suboptimal concentrations in the fractionated cell-free system.

An apparent analogue of Krebs ascites $\mathrm{IF_{EMC}}$ termed IF-E4 (ref. 2) or IF-M4 (ref. 13), has been recognised in rabbit reticulocytes, and is similar to $\mathrm{IF_{EMC}}$ in molecular weight and chromatographic behaviour. We therefore compared the requirement for $\mathrm{IF_{EMC}}$ and IF-E4 in EMC RNA translation in the $\mathrm{IF_{EMC}}$-deficient ascites cell-free system (Fig. 1). $\mathrm{IF_{EMC}}$ and IF-E4 were equally efficient in stimulating EMC RNA translation at saturating factor concentration. The greater specific activity shown by IF-E4 presumably reflects the higher purity of this factor compared with $\mathrm{IF_{EMC}}$.

We then examined separately the specificity of IF-E4 in the translation of several mRNAs in the ascites cell-free system (Table 2). The results correspond closely to those obtained with $\mathrm{IF_{EMC}}$ (Table 1). EMC RNA, mengo virus RNA and foot-and-mouth disease virus RNA require the

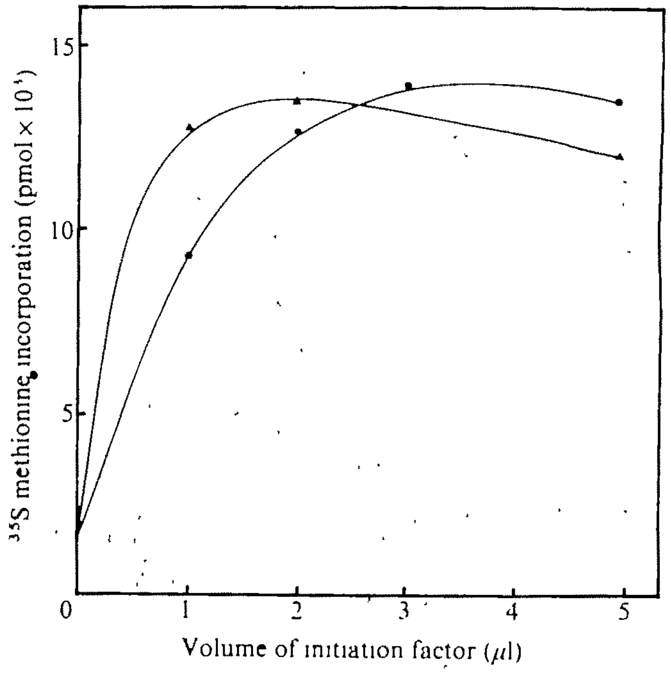

Fig. 1 Dependence of EMC RNA translation in the ascites fractionated cell-free system on $\mathrm{IF_{EMC}}$ (from Krebs ascites cells) (●) and IF-E4 (from rabbit reticulocytes) (▲). Protein synthesis assays were performed as described in Table 1, containing 0.02 A_{260} units EMC RNA and increasing volumes of $\mathrm{IF_{EMC}}$ (protein concentration 1 mg ml^{-1}) or IF-E4 (protein concentration approximately 0.3 mg ml^{-1}). Incorporation in the presence of EMC RNA was corrected for a value of 0.0094 pmol ^{35}S-methionine obtained in the absence of added EMC RNA. No alteration in this background value was observed on addition of either initiation factor. 1 pmol $\approx 26 \cdot 10^4$ c.p.m.

Table 1 Effect of the addition of Krebs ascites cell $\mathrm{IF_{EMC}}$ on the translation of various mRNAs in the fractionated ascites cell-free system

mRNA	^{35}S-methionine incorporation (pmol × 10^3) Control	 Control + $\mathrm{IF_{EMC}}$	Stimulation by $\mathrm{IF_{EMC}}$ (% control value)
Encephalomyocarditis virus	7	55	686
Mengo virus	5	88	1,660
Foot-and-mouth disease virus	18	62	233
Polio virus	11	10	0
Semliki forest virus (42S RNA)	15	12	0
Semliki forest virus (26S RNA)	12	18	50
Vaccinia virus *in vitro* mRNA	26	27	4
Tobacco mosaic virus	42	44	5
Alfalfa mosaic virus	17	17	0
Brome mosaic virus	11	13	18
Satellite tobacco necrosis virus	34	48	37
Tobacco rattle virus	45	56	24
Haemoglobin mRNA	10	14	40
Oviduct polysomal RNA	12	10	0

The components of the fractionated ascites cell-free system were prepared as previously described[9]. Protein synthesis assays were performed in 25-µl reaction mixtures containing 0.45 A_{260} units ascites ribosomes, 5 µg elongation factor 1, 0.02 A_{260} units ^{35}S-methionine–tRNA (0.8 pmol ^{35}S-methionine), 1–6 µg mRNA, 1–2 µg $\mathrm{IF_{EMC}}$, 20 mM Tris-HCl (pH 7.5), 3.5 mM $MgCl_2$, 100 mM KCl, 6 mM 2-mercaptoethanol, 1 mM ATP, 0.1 mM GTP, 8 mM creatine phosphate and 100 µg creatine phosphokinase. The ionic conditions were optimal for several mRNAs tested, that is, EMC RNA, Semliki forest virus 26S RNA and tobacco mosaic virus RNA. $\mathrm{IF_{EMC}}$ (purified to the Sephadex G-100 stage[8]) and each mRNA was added in saturating concentration. Incubation was at 37 °C for 30 min. Hot trichloroacetic acid-precipitable radioactivity was measured as previously described[9]. Incorporations in the presence of mRNA were corrected for background incorporation of 0.010 pmol ^{35}S-methionine in the absence of added mRNA. 1 pmol ^{35}S-methionine is approximately equivalent to 25 10^4 c.p.m.

Table 2 Effect of the addition of rabbit reticulocyte IF-E4 on the translation of various mRNAs in the fractionated ascites cell-free system

mRNA	^{35}S-methionine incorporation (pmol × 10^{3}) Control	Control × IF-E4	Stimulation by IF-E4 (% control value)
Encephalomyocarditis virus	28	142	410
Mengo virus	28	159	470
Foot-and-mouth disease virus	51	222	335
Polio virus	48	52	8
Vaccinia virus *in vitro* mRNA	46	48	4
Tobacco mosaic virus	178	179	0
Brome mosaic virus	62	80	3
Tobacco rattle virus	168	177	5
Satellite tobacco necrosis virus	111	126	14
Haemoglobin mRNA	14	22	57
Oviduct polysomal RNA	39	52	33

Protein synthesis directed by each mRNA was assayed at saturating mRNA concentration as described in Table 1 and the effect of the addition of a saturating amount of IF-E4 was tested. Incorporations in the presence of mRNA were corrected for background incorporation of 0.015 pmol ^{35}S-methionine in the absence of added mRNA. 1 pmol ^{35}S-methionine is ≈ 19 × 10^{4} c.p.m.

addition of IF-E4 for translation, whereas the remainder of the mRNAs tested show either no significant dependence (for example, tobacco mosaic virus RNA) or a partial dependence (for example, oviduct polysomal RNA) on IF-E4 for translation. The optimal concentration of monovalent and divalent cations for EMC RNA translation in the presence of either IF$_{\mathrm{EMC}}$ or IF-E4 was tested and found to be the same for either factor, that is, optimal translation was obtained at 100 mM KCl and 3.5 mM MgCl$_2$. In common with IF$_{\mathrm{EMC}}$, IF-E4 is required for the synthesis of EMC-specific initiation peptides formed in the presence of sparsomycin[14], an inhibitor of polypeptide elongation, suggesting that both factors act at the level of initiation of protein synthesis.

Staehelin's group has shown that IF-E4 is required for the translation of both globin mRNA and EMC RNA in a completely fractionated protein-synthesising system[2]. Moreover, a twofold higher concentration of IF-E4 was required for optimal EMC RNA translation than for globin mRNA translation. Because EMC RNA is larger than globin mRNA, in molar terms this represents a greater IF-E4 requirement for EMC RNA translation, suggesting that the amount of IF-E4 required for optimal translation varies according to the mRNA tested. Our results are consistent with these findings. The high requirement for IF$_{\mathrm{EMC}}$ in EMC RNA translation is expressed by the need to add exogenous factor to the fractionated ascites cell-free system for translation to proceed. A low level of IF$_{\mathrm{EMC}}$ contamination of the ascites cell-free system could explain the absence of dependence or partial dependence on IF$_{\mathrm{EMC}}$ for the translation of several mRNAs (Table 1). In support of this idea, we have detected IF$_{\mathrm{EMC}}$ activity in a 350 mM KCl wash of the ribosomes used in the standard protein synthesis assay. We were unable to prepare ribosomes devoid of IF$_{\mathrm{EMC}}$ which retained good activity in natural mRNA translation in the fractionated cell-free system.

Hellerman and Shafritz have suggested that certain initiation factors bind to mRNA[15]. Thus both the Met-tRNA$_{\mathrm{f}}$ binding factor and messenger ribonucleoproteins (mRNPs) from rabbit reticulocytes bound to globin mRNA and poly(A). A polypeptide of molecular weight 52,000 was found both in the initiation factor and the mRNP preparation. In spite of the close similarity in molecular weight of this polypeptide to IF$_{\mathrm{EMC}}$, we could not demonstrate co-electrophoresis in sodium dodecylsulphate (SDS) gels of IF$_{\mathrm{EMC}}$ with any of the proteins of ascites mRNPs. This suggests that IF$_{\mathrm{EMC}}$ does not form a stable association with mRNA.

We conclude that IF$_{\mathrm{EMC}}$ and IF-E4 are functionally identical and have analogous roles in the initiation of protein synthesis in rabbit reticulocytes and mouse ascites cells. This factor is therefore not specific to cell, tissue or species but may be required in variable amounts for the translation of all mRNAs. It is interesting that variable amounts of the prokaryotic initiation factor IF3 are needed for the translation of different bacterial mRNAs[16]. IF3 may stabilise the direct base-pairing between the 5′ terminus of mRNA and the 3′ end of 16S ribosomal RNA shown to be important in the initiation of prokaryotic protein synthesis[17]. We speculate that IF$_{\mathrm{EMC}}$ and IF-E4 effect a similar stabilisation in eukaryotic mRNA–ribosome interaction[18].

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A search for *in vivo* factors in regulation of microtubule assembly

MICROTUBULES are abundant in eukaryotic cells where they assume various functions[1], some of which seem to be at a purely structural level, such as maintenance of cell shape and axonal elongation, whereas others are involved in more dynamic processes, such as mitosis, meiosis, secretion and axonal transport. At least two distinct protein factors of

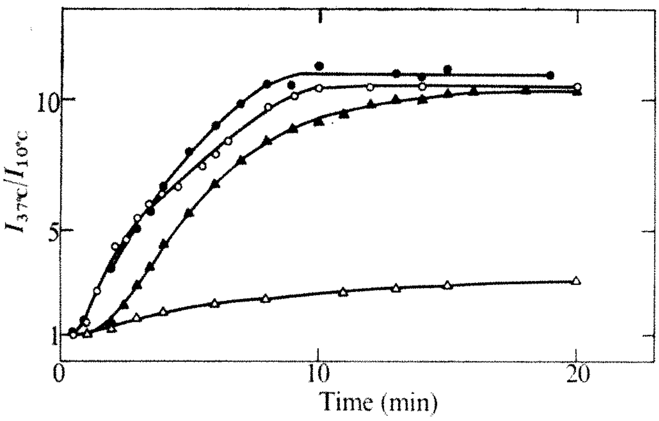

Fig. 1 For preparation of microtubules, rat brains were homogenised in 2 volumes of MES buffer and microtubules polymerised from the 105,000*g* supernatant using the method of Shelanski *et al*[11]. The pellets obtained after centrifugation for 30 min at 50,000 r.p.m. and 25 °C, in a Spinco 50 rotor, were washed, and after resuspension, depolymerised for 30 min at 0 °C. The supernatant obtained after 30 min of centrifugation at 40,000 r.p.m. and 0 °C, in a Beckman 50 rotor, was used to analyse the kinetics of microtubule selfassembly by light scattering, electron microscopy and analytical ultracentrifugation. The rate of polymerisation of brain homogenates was followed by monitoring the time dependence of their scattering at 37 °C at an angle of 90° in a Fica light scattering photometer. The ratio of scattering at 37 °C to that at 10 °C (I_{37}/I_{10}), reflecting the polymerisation process was measured in the absence (open symbols) and presence of glycerol (solid symbols) on brain homogenates from 5-d-old (△) and 30-d-old rats (○). Similar scattering patterns were observed for solutions obtained after a one-cycle polymerisation purification. At 10 °C no change in scattering of solutions was detected during the experiments.

different molecular weight have been suggested[2-5] to regulate microtubule assembly. The uniqueness of their role is not unequivocally established because microtubules have also been obtained in the absence of these protein factors, at high magnesium ion concentrations[6], in the presence of polycations[7] or glycerol[11]. To correlate better the results obtained *in vitro* with the mechanism operating *in vivo*, we have studied the assembly of microtubules in extracts prepared from developing rat brain, differentiating mouse neuroblastoma cells and in chicken sensory ganglia, which may facilitate detection of a factor which induces self-assembly *in vivo*. We conclude that the high molecular weight protein is seemingly not required for the *in vivo* assembly of microtubules.

The rate and extent of polymerisation of microtubules, as measured by light scattering, in soluble extracts prepared from brains of 5-d-old rats was much lower than in extracts from 30-d-old rat brains (Fig. 1). This confirms observations made by Fellous *et al.*[9-10]. Glycerol promotes tubulin assembly[11], and in the presence of glycerol the polymerisation rate of the extract of 5-d-old brain was considerably increased, reaching a level comparable with that of the 30-d-old extract. Depolymerised microtubules prepared from 5-d-old rat brains did not show the ring component in the analytical centrifuge (36S) that was present in extracts from adult animals (Fig. 2). Electron micrographs of these extracts confirm that rings were present only in the 30-d extract. Our measurements of the 'ring' peak indicate a sedimentation coefficient of 30–33S. Thus, as our preparation procedures were similar to those of Shelanski *et al.*[11], we conclude that we are dealing with the native tubulin ring sedimenting at 36S, rather than the 42S ring component whose formation was induced by a high magnesium level (see ref. 8, page 4565).

These data raise two main questions: are the microtubules formed in the presence of glycerol similar to those polymerised in its absence, and is there a correlation between the higher rate of polymerisation of microtubules in extracts from mature brains and the presence of 'rings' and other protein species absent from extracts of younger animals? We have observed that microtubules polymerised in the presence or absence of glycerol have the same gross morphology and optical diffraction patterns of their micrograps display identical periodicities. Thus with respect to periodic features the two species are indistinguishable. It should be noted, however, that microtubules polymerised in the presence of glycerol have been reported to contain different amounts of accessory proteins. The presence of glycerol also leads to the formation of rings of apparently different shape or subunit composition, as reflected by their sedimentation coefficients[12].

Rings cannot be formed from purified 6S tubulin, but they can be assembled in the presence of a high molecular weight protein which has yet to be fully characterised[3-5] or in the presence of high magnesium concentration[8], albeit with apparent change in their structure in the latter case. To test for the presence of this protein factor, extracts were analysed by electrophoresis on SDS–polyacrylamide gels (Fig. 3). There was no clear correlation between the relative content of high molecular weight protein in the extract and the speed of polymerisation, and the relative amount of this protein in microtubules did not correlate with the animal's age. These results suggest that the high molecular weight protein, although able to induce *in vitro* microtubule assembly, is not required for *in vivo* polymerisation. In line with this conclusion is our inability to detect high molecular weight protein by autoradiography of sodium dodecyl sulphate (SDS)–polyacrylamide gels of microtubules from differentiating neuroblastoma cells[13] or sensory ganglia exposed to nerve growth factor (unpublished observations by H. Schmitt and S. Mizel), labelled with ^{3}H-leucine or ^{35}S-methionine.

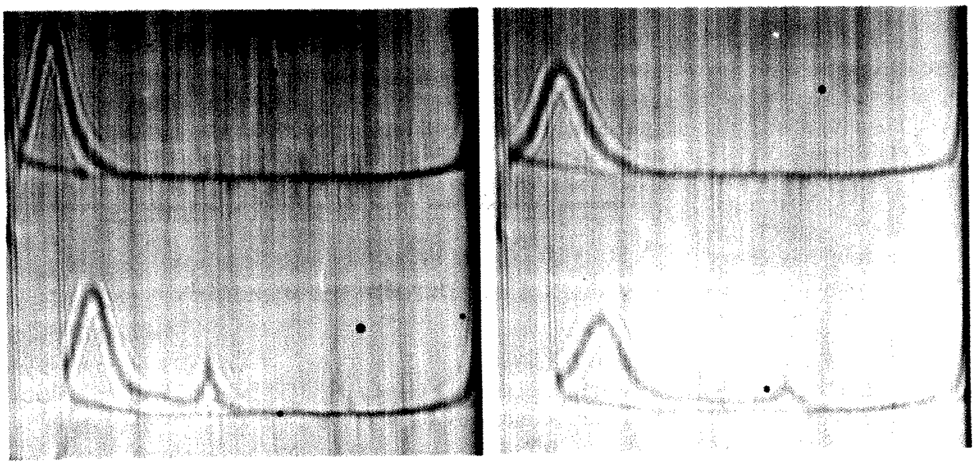

Fig. 2 Schlieren pattern of tubulin sedimenting in the ultracentrifuge at 44,000 r.p.m. and 7 °C. The upper cell contained tubulin from 5-d-old rat brain, and the lower cell, tubulin from 30-d-old rat brain. The first pattern was obtained after 16 min and the second pattern after 32 min of sedimentation. The upper pattern has only a single 6S peak and the lower cell has a 6S and a 36S peak. Electron microscopy confirms that the 36S component consists of rings.

On the other hand, a protein with a molecular weight, on SDS–polyacrylamide gels of 82,000 was reproducibly found in microtubules prepared from brains of rats older than 10 d and not from younger animals (Fig. 3). The molecular weight of this protein is similar to that of τ factor, first described by Weingarten *et al.*[5,14,15]. This observation supports the report by Fellous *et al.*[10] that partially purified τ factor enhances the rate of polymerisation of microtubules from extracts of young rat brains and raises the possibility that it interacts with tubulin and facilitates polymerisation. A more definitive indication of the role of this protein will be obtained if it can be shown to be associated with microtubules polymerising *in vivo.*

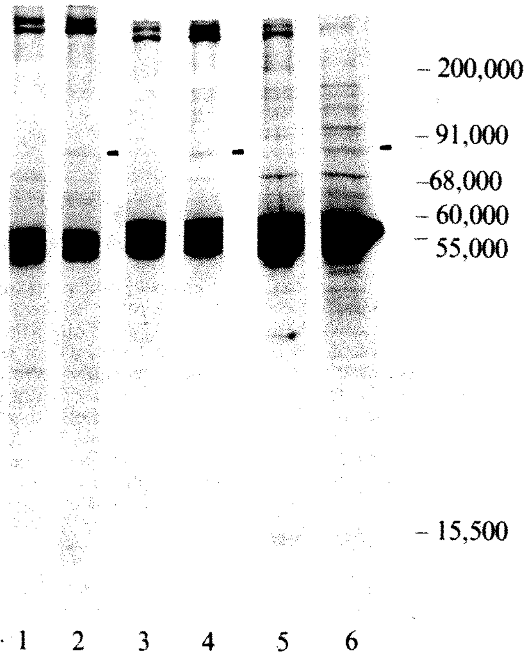

Fig. 3 SDS–polyacrylamide gel analysis of polymerised microtubules from 5- and 30-d-old rat brains. Microtubules were purified by one-cycle of self-assembly as described in Fig. 1. Pellets were resuspended and analysed on 10–20% polyacrylamide slab gels containing 0.1% SDS, as previously described[13]. Markers used to calibrate the gels included myosin (200,000), phosphorylase (91,000), bovine serum albumin (68,000), catalase (60,000), tubulin (55,000) and globin (15,500). 1, 3 and 5 are microtubules purified from 5-d-old rat brains and 2, 4 and 6 are from 30-d-old rat brains.

Our finding that extracts from young rat brains which do not display ring formations polymerise in the presence of glycerol, to yield microtubules similar to those of adult rat brain extracts in which the rings are abundant raises questions as to the validity of extrapolation of *in vitro* studies to the *in vivo* mechanism. Involvement of a membrane-bound initiator protein, with a function similar to that of α-actinin in actin polymerisation, cannot yet be discounted. Such a mechanism could explain the exclusive localisation of microtubules in the growing neurites of the nerve cell.

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Tubulin hoops

CYTOPLASMIC tubulin displays a wide range of polymorphism, including small sheets[1] and rings or spirals[2]. We report here several new structures. One of the most interesting aggregates is a hoop which has a triplet substructure and arrangement of the tubulin dimer, similar to that found in flagellar B tubules.

Tubulin hoops were formed by polymerisation of purified protein in the conditions listed in the legend to Fig. 1. Initially, the preparation contained narrow, long, wavy sheets (about 3–7 protofilaments wide). If allowed to polymerise further, the sheets became wider (between 10 and 15 protofilaments) and straightened out. They sometimes folded into microtubules (13 protofilaments) or associated laterally with other sheets oppositely oriented; other sheets folded into small tubules (8–11 protofilaments). Narrow sheets often curled into hoops of 1,000–1,400 nm in circumference. The hoops are thus shallow spirals, and these grew up to widths of 400 nm (about 80 protofilaments). Depending on how they settled down on the carbon film, different images were obtained (Figs 1, 2).

The outside of the protofilaments in the hoops corresponds to the inside of the protofilaments in microtubules. This is evident from Fig. 2*b*, where the inner portions of a hoop lie flat on the carbon film, but adjacent stretches do not fold over properly, as in Fig. 2*a*. The resulting stress leads to breaks or distortions at the corners on the inner side, while the outer rim stays elevated, as verified using platinum shadowed specimens. These images indicate that the outside of the hoop lies on the grid; and by correlating the observed striations with those of the left-handed microtubule helix[3], we deduce that the protofilaments in the hoop are turned inside out relative to the microtubule.

Optical diffraction patterns of the hoop images are best understood in terms of the surface lattice of microtubules[4] or sheets[1]. In Fig. 3*a* the lines connecting the lattice points apply to a microtubule viewed from the front; the back would have the same spacings, but opposite inclinations. The diffraction spots expected from this lattice are asymmetric with respect to the vertical axis if we see either front or back (for example, in sheets, or hoops opened up on the microscope grid (Fig. 2*a*)), and symmetric when front and back are superimposed, such as in microtubules or hoops viewed from the side (Fig. 1).

Some immediate inferences from optical diffraction patterns are that the hoop side view (Fig. 1) is a two-layered structure showing front and back (spots symmetric about the meridian), that the top views are one layered (asymmetric spots), and that adjacent stretches in a proper top view face opposite ways (Fig. 2*a*), but in the same direction in a partial top view (Fig. 2*b*). The spacing of the first layer line is highly reproducible (within 1%), $c = 4$ nm in all our polymorphic aggregates, showing the protofilaments to be connected longitudinally along nearly invariant bonds[5]. The lateral separation between protofilaments varies in the hoops but averages 4.7 nm; this corresponds to the separation of the protofilaments near the

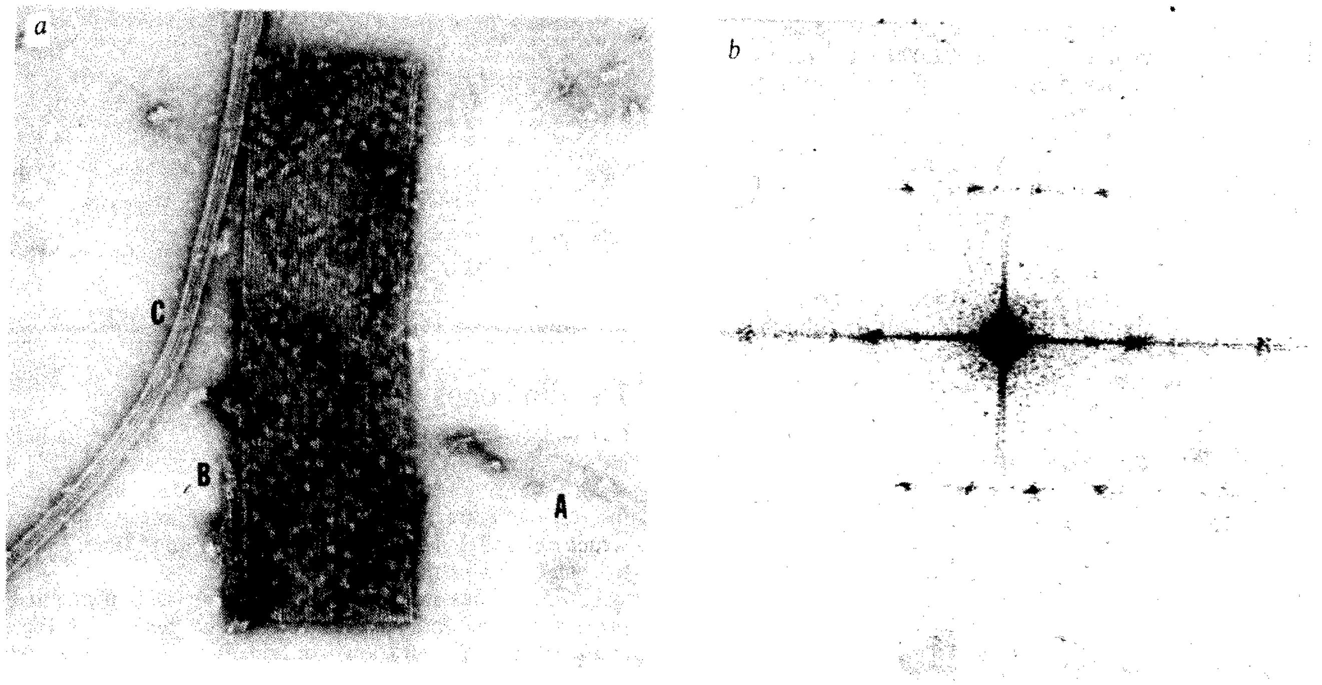

Fig. 1 *a*, Hoop viewed from the side, containing about 40 protofilaments. The probable parent sheet is seen at A, the growth point protruding from the hoop at B. C is a minitubule made from nine protofilaments (× 140,000) Tubulin, prepared essentially according to Shelanski *et al.*[11], was purified by three cycles of polymerisation in reassembly buffer (0.1 M MES, 1 mM EGTA, 1 mM GTP, 1 mM DTT, 0.5 mM $MgCl_2$, *p*H 6.5 at 4 °C) with the addition of 25% glycerol. The protein (5–10 mg ml^{-1} in cold reassembly buffer), was diluted to 1 mg ml^{-1} or less, by adding warm (25 °C, *p*H 6.5, 10–25% glycerol) buffer. After 1–2 min the temperature was raised to 37 °C and polymerisation followed for up to 1 h. Samples for electron microscopy were removed at various intervals, placed on carbon support grids, stained with 1% uranylformate, and examined in a Philips EM 301 electron microscope. Conditions for minimising electron dose were as described elsewhere[12]. *b*, Diffraction pattern of (a). The strong spots on the equator (using the notation of Fig. 3) are the (13, 1) at a spacing of 4.8 nm and (26, 1) at 2.4 nm. Ghost reflections arising from tipling occur at orders of 14.2 nm out to a resolution of 2 nm. The 4-nm layer line is dominated by 4 spots symmetric about the meridian, pairs of (−3, 1) and (10, 1) reflections from front and back, with weak ghosts in between. The 2-nm layer line shows a pair of spots, index (7, 2). Very weak reflections on the 8-nm layer line lie midway between the origin and the (−3, 1) spots. This shows that the arrangement of dimers in the hoops is similar to that in flagellar B subfibres (see text).

centre of the microtubule wall[6]. The shift between adjacent protofilaments also varies and is usually greater than 0.9 nm. Thus, in most cases, 13 adjacent protofilaments cannot be wrapped into a closed microtubule, as is possible with Erickson's sheets[1]. The surface lattice does, however, often fit one of the minitubules mentioned above.

A striking aspect of the images is the periodic tripling of protofilaments. Correspondingly, optical diffraction

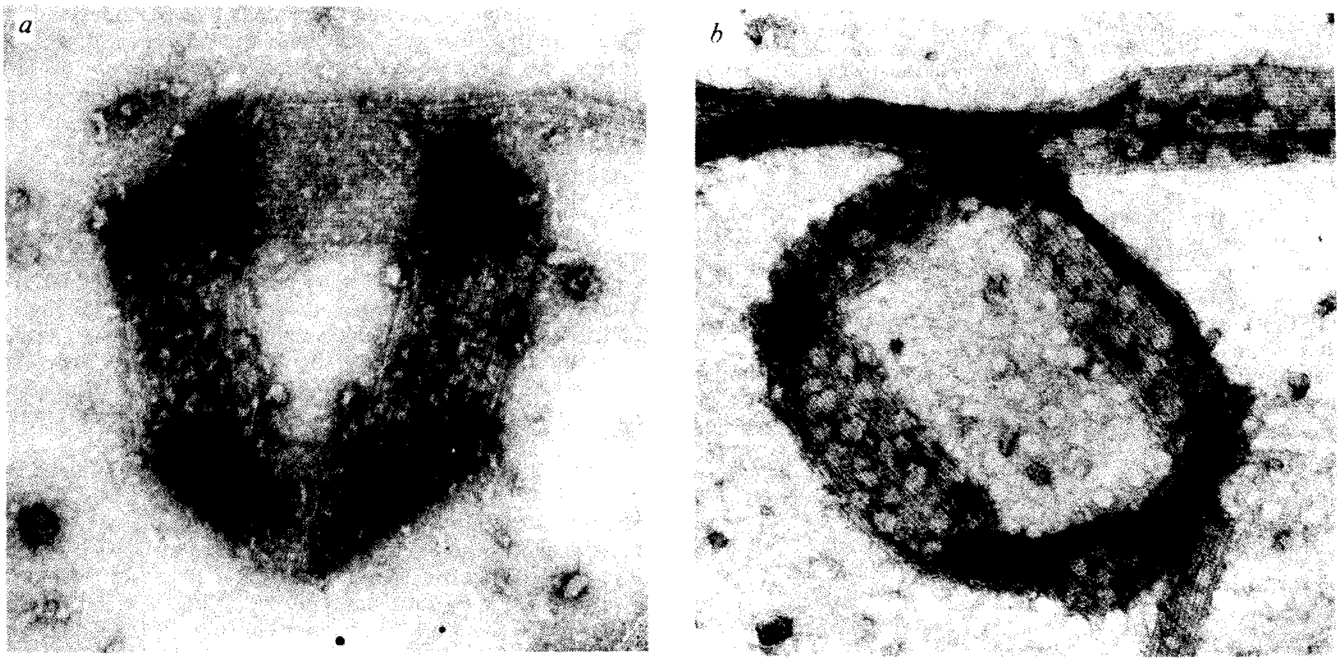

Fig. 2 *a*, Hoop opened up with all sides flattened on the grid. Adjacent stretches are alternating up and down (× 140,000). *b*, Hoop with adjacent stretches facing the same way up. The outer rim is folded over, away from the grid, as established by platinum shadowed specimens. From the inclinations of the (−3, 1) striations it follows that the outside of the hoop, which faces the carbon, corresponds to the inside of the microtubules. This conclusion is supported by the fact that the (−3, 1) striations in the hoop and those in the adjacent sheet which is folding into a tubule, are of opposite inclinations (× 140,000).

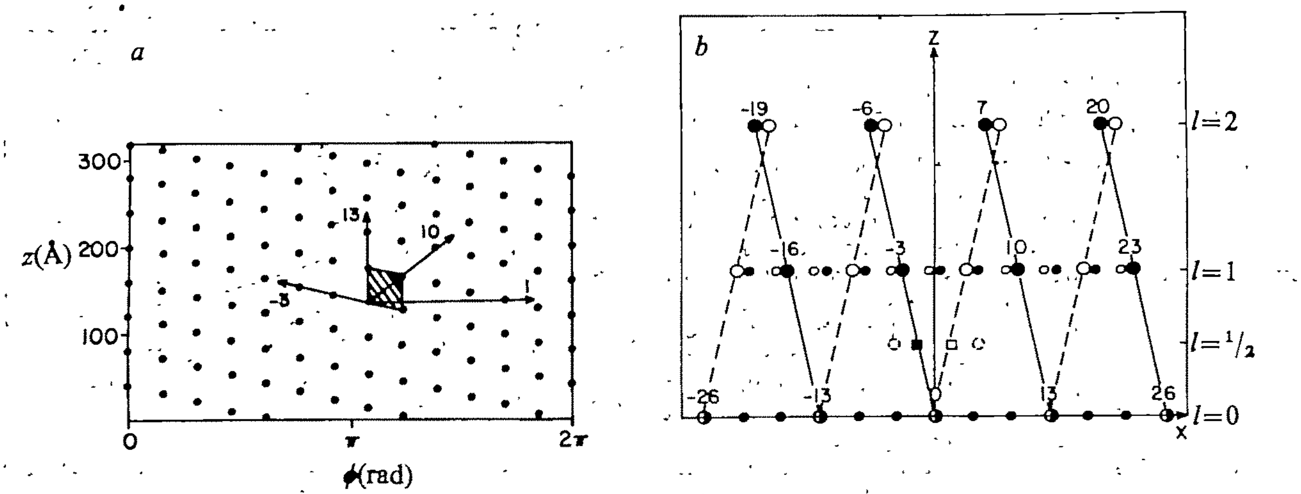

Fig. 3 *a* Microtubule surface lattice according to Amos and Klug[4] as viewed from outside. Directions of main helical families are indicated. There are 13 vertical protofilaments, staggered by 0.9 nm to produce a left-handed 3-start or a right-handed 10-start helix. The 1-start genetic helix does not connect adjacent subunits, so is of no physical significance. Area of unit cell is shaded. *b*, Diffraction pattern expected for flat (two-dimensional) sheets of protofilaments. Microtubule helix nomenclature is used to characterise the diffraction spots: (n, l) refers to the number of helices in a given family, l is the layer line indicating their vertical separation, c/l (c = 4 nm). The equatorial spot (13, 0) comes from the lateral separation of protofilaments; (26, 0) is the second order showing a deepening of the groove and/or a protofilament splitting[1,8]. The (−3, 1) spot on the first layer line arises from the shallow left-handed 3-start helix which is relatively enhanced in electron images, while the (10, 1) spot corresponds to the steeper 10-start helix dominating the X-ray pattern. Open and filled circles correspond to the sheet facing up or down. The spacings of spots in the two lattices (solid and dotted lines) are the same, but inclinations are symmetric about the meridian (Z-axis). Sheets or hoop top views have either open or filled reflections, hoop side views have both. On the equator (l = 0) both lattices are superimposed. The ghost reflections arising from tripling are indicated only on the equator and the first layer line. Numbers refer to the rotational symmetry n of the corresponding structural feature in the microtubule. The quadratic spots on l = 1/2 indicate the B-type superlattice found in hoops; the dotted circles show the positions expected for an A-type bonding (not observed). Hoops are wound up from shallow spirals of parent sheets so that in side views the solid and dotted lattices are slightly rotated (1–2°) with respect to one another.

patterns show a series of ghost reflections dividing the space between main spots into three parts along the horizontal direction (Fig. 1*b*). Hoops smaller than about 10 protofilaments do not show tripling in a systematic manner, nor do the sheets, although some separation into triplets can occasionally be found. In particular, parent sheets forming hoops usually contain between three and seven protofilaments, not only three or multiples thereof. Tripling thus seems to represent an inherent bonding property of cytoplasmic tubulin.

The physical basis for tripling is not known. Flagellar outer doublet microtubules have a partition wall between A and B tubules consisting of three protofilaments of particular stability[7]. Their coherence is attributed to extra protein components[8]. It is possible that even cytoplasmic tubulin has some propensity to form a 'partition wall'. A second inference comes from duplex tubules[9]; that is, cytoplasmic microtubules coated with an outer layer of protofilaments running along a flat helix. The diffraction pattern shows a 15-nm layer line which could mean that the duplex helix repeats after three turns or that the turns are grouped together in threes.

Another unusual bonding property of the hoops is indicated by a very weak 8-nm layer line (Fig. 1*b*). In their analysis of flagellar microtubules, Amos and Klug[4] derive two different surface lattices for the positions of α-β-heterodimers. In both cases the dimers are assumed to lie along the protofilaments ($\alpha\beta\alpha\beta$ . . .). Outer doublet A tubules have alternating subunits along the 3-start helices ($\alpha\beta\alpha\beta$ . . .). Thus along the 10-start helices the bonding is either ($\alpha\alpha\alpha$ . . .) or ($\beta\beta\beta$ . . .). For the incomplete B tubules the reverse holds true. These structures are distinguished by the positions of reflections on the 8-mm layer line (Fig. 3*b*). This analysis suggests that the arrangement of dimers in the hoops is similar to that in the flagellar B subfibres.

Identical bonding of dimers, assuming a 13-protofilament microtubule[10] is possible only with the A-type subunit arrangement. Symmetry arguments therefore suggest that cytoplasmic microtubules have A-type bonding. But, the B-type packing observed with hoops, if present in microtubules, would imply non-identical bonding of subunits. X-ray patterns from microtubules support this possibility[6]. Thus, both the tripling of protofilaments and the B-type lattice in the hoopes suggests a close relation between cytoplasmic and flagellar tubulins.

These studies were undertaken to obtain large, well ordered aggregates of tubulin to aid in the interpretation of X-ray patterns[6]. The hoops contain many unit cells (up to 20,000), and even when negatively stained, their diffraction patterns usually extend to the second and occasionally to the third layer line. This should make them useful in extending the resolution of the existing microtubule model.

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Comparison of bacterial and animal rhodopsins by hydrogen exchange studies

RHODOPSIN is deeply embedded in a hydrocarbon membrane, yet hydrogen exchange (HX) studies show that about 70% of its peptide hydrogens are freely exposed to solvent water[1]. This contrasts with other membrane-bound proteins and even aqueous proteins which generally involve about 70% of their peptide hydrogens in internal H-bonding. It has been suggested that the large fraction of disordered polypeptide chain indicated for rhodopsin resides in and acts to stabilise a channel of water penetrating into the membrane, and further that this channel must be quite large, perhaps 12 Å or more in diameter[1]. Unwin and Henderson[2] described an electron diffraction model for the purple membrane protein of *Halobacterium halobium* (bacteriorhodopsin) which compares interestingly with the suggested structure of animal rhodopsin; membrane-embedded bacteriorhodopsin is arranged in a three-molecule circle surrounding an open space 20 Å in diameter. This space, however, is filled not with water but with lipid, and the protein walls of this blind channel are formed of a double rank of α helices which account for most of the bacteriorhodopsin polypeptide chain, so that bacteriorhodopsin, unlike animal rhodopsin, must be as extensively H-bonded as is the usual protein. We report here a HX study of bacteriorhodopsin. In agreement with the electron diffraction model, our HX results show that this protein, unlike animal rhodopsin, has about 75% of its peptides internally H-bonded. These and previous results, however, suggest a fundamental structural similarity between the two rhodopsins.

HX curves for bacteriorhodopsin in purple membrane are shown in Fig. 1. The slowly exchanging hydrogens measured come from internally H-bonded peptide groups of the membrane-bound protein (for discussion see ref. 1) To measure these, they were initially labelled, before the exchange-out experiment started, by incubating membrane suspensions in tritiated water for long periods at high *p*H to promote fast exchange Exchange of the slow hydrogens that contribute to Fig. 1 was not characterised in any detail because we are more interested at this stage in the number of freely exposed peptide hydrogens in bacteriorhodopsin in order to compare this with the diffraction model and with animal rhodopsin.

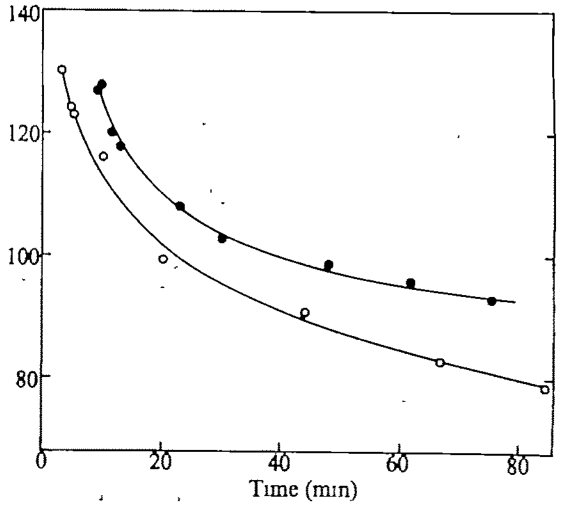


Fig. 1 Hydrogen–tritium exchange-out data for purple membrane suspensions at *p*H 5.0 (○) and *p*H 4.7 (●). The bacteria were grown and the membranes were isolated according to Osterhelt and Stoeckenius[10]. After exchange-in at *p*H 10.5 (2 mM alanine buffer) for 5 d at 4 °C, the purple membrane suspension was adjusted to the appropriate *p*H and passed through a medium Sephadex-G25 column (at low hydrostatic pressure; 0 °C; *p*H 5.0, 10 mM cacodylate or *p*H 4.7, 5 mM citrate) to remove free tritiated water and initiate exchange-out. HX methods were detailed elsewhere[11]. Two to three drop fractions collected from the final columns were diluted to 0.5 ml with 0.1 M phosphate buffer at *p*H 7.4, and maintained at 0 °C in order to measure absorbance. (Absorbance measurements decrease slowly with time at room temperature and are slightly dependent upon *p*H and salt concentration.) Protein concentration in the samples was then computed using a molar extinction coefficient of 7.2×10^4 at 570 nm, determined using the retinaloxime method of Wald and Brown[12] and the thiobarbituric acid method of Futterman[13]. (Total range on 4 preparations was $7.0–7.5\times10^4$.) Bound tritium in the samples was assayed by liquid scintillation counting. These data were used to compute the number of hydrogens per molecule still unexchanged as a function of exchange-out time.

To measure the faster exchanging, freely exposed peptide hydrogens, the earliest part of the curves in Fig. 1 must be studied. It is difficult to measure with good resolution these faster hydrogens above the large background of slow hydrogens High resolution can be achieved by use of a limited exchange-in manipulation, described before (for example, ref. 3), which suppresses the background of slow hydrogens. Samples at *p*H 5 and 0 °C, where the free peptide half-time is close to 1 min, were initially exchanged-in for only 5 min, then quickly passed through a Sephadex column to terminate labelling and initiate exchange-out. This limited incubation period labels all the free peptides but only a greatly reduced number of the more slowly exchanging sites. The measured exchange-out data for the partially labelled protein (Fig. 2*a*) are fitted by a fast phase with the expected free peptide half-time and a slower phase with no predetermined parameters. The isolated fast phase is shown in Fig. 2*b*. Essentially the same procedure was repeated at *p*H 4.7 where peptide HX rate is twofold slower (Fig. 3). In both cases the data indicate ~ 50 hydrogens per molecule exchanging at the expected free peptide rate. One can add 10 prolines to the unbonded residues. These amount to 25% of the total of 242 peptide groups[4] in the molecule. This value is in line with the small fraction of free peptide hydrogens generally seen in other proteins and is consistent with the small fraction expected from the Unwin–Henderson model[2], but it is very different from the value of ~70% free peptides found for animal rhodopsin

The total number of hydrogens measured in these experiments can be obtained with best accuracy by considering Figs 1 and 2 together. Figure 2*a* gives the number that exchange in 5 min at *p*H 5.0 (since initial exchange-in time was 5 min) The number of hydrogens that exchange between 5 min (*p*H 5) and very much longer times can be obtained from the 5-min point on the *p*H 5.0 curve of

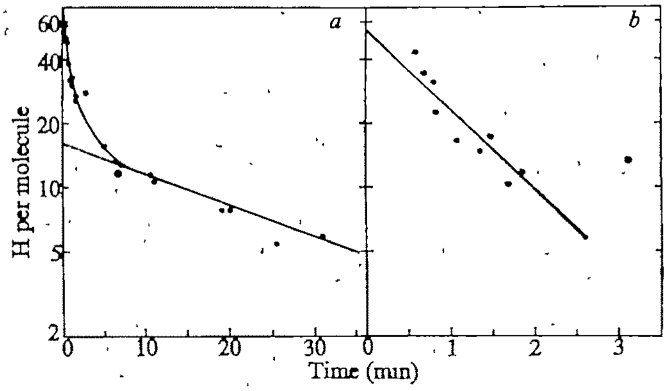
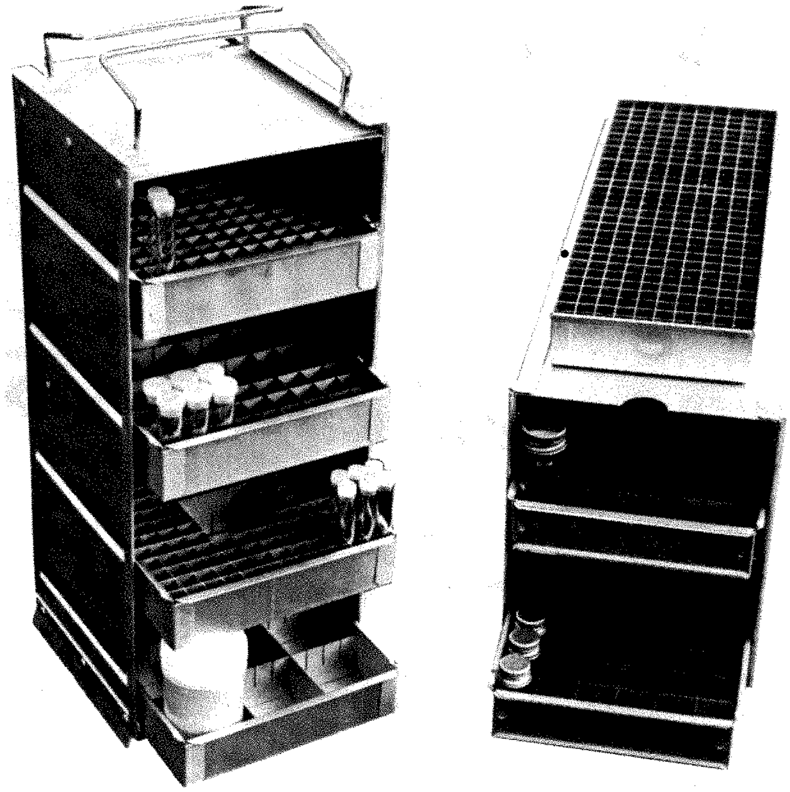
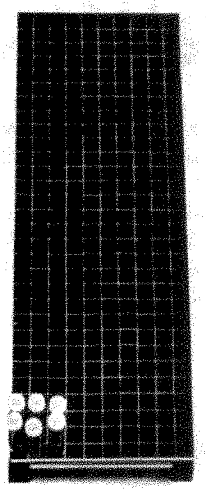
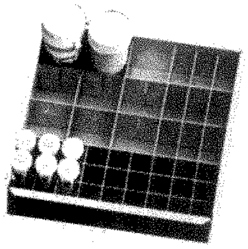


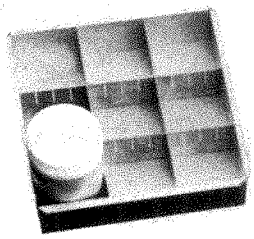
Fig. 2 Limited exchange-in/exchange-out data at *p*H 5.0 and 0 °C. Purple membrane suspensions were labelled at 0 °C for only 5 min at *p*H 5 (10 mM cacodylate) and then passed quickly through a Sephadex column to initiate exchange-out. A semilog plot of the resulting data is given in *a* and the isolated free peptide phase, obtained by subtracting the slow phase from the early time data, is in *b*.

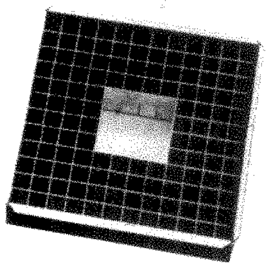
ADDING A NEW DIMENSION TO LUCKHAM LAB TRAYS... AND FREEZFILES.











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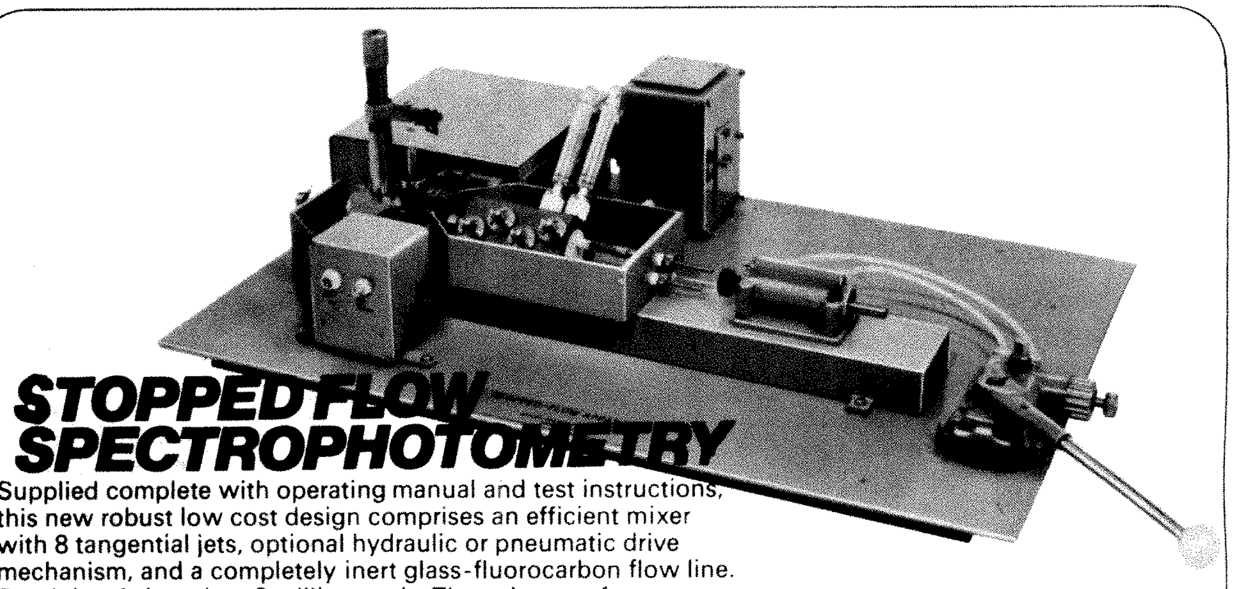

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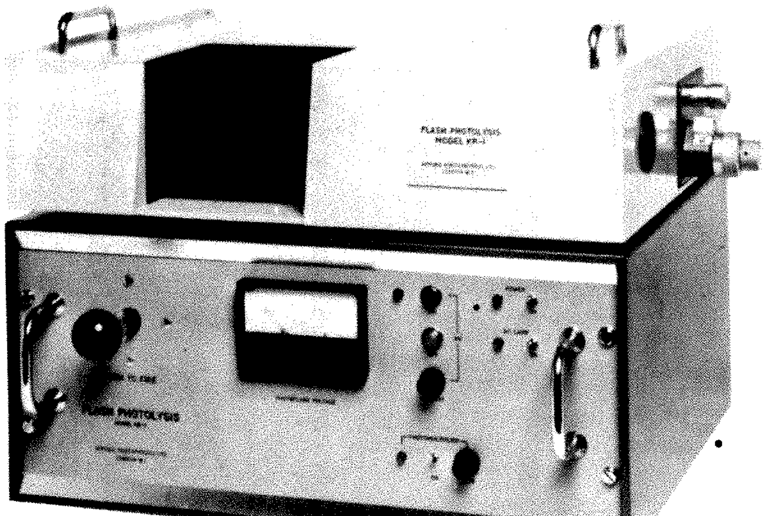

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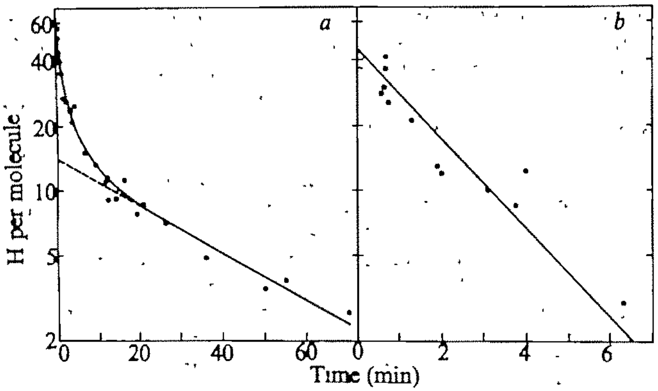

Fig. 3 Limited exchange-out data at pH 4.7 and 0 °C. Exchange-in was for 10 min at pH 4.7 (5 mM citrate) and subsequent treatment was as for Fig. 2.

Fig. 1. The sum of the faster and the slower hydrogens then yields the total number. Similarly, an independent estimate can be obtained from the pH 4.7 data (Figs 1 and 3a). The total number found, ~190 per bacteriorhodopsin molecule, is significantly lower even than the total number of amide hydrogens present (232 peptide H plus from 0 to 64 side chain amide H[4,5]; degree of amidation in unknown). In similar studies with other proteins including membrane-bound animal rhodopsin, the number of hydrogens measured was experimentally identical with the number of amide hydrogens present. The resistant sites in bacteriorhodopsin which could not be labelled by the rigorous exchange-in conditions used here seem to carry the most slowly exchanging peptide hydrogens as yet indicated for any protein, with an exchange rate slower than the expected free peptide rate by a factor of 10^{10} or more. In view of the structure and placement of bacteriorhodopsin, this result is perhaps not too surprising.

These results indicate that bacteriorhodopsin has about 60 free peptides and therefore 180 internally H-bonded ones. This compares with the minimum number of 150 peptide H-bonds required by the Unwin–Henderson model[2] (to generate seven helices of seven turns each with the final turn non-bonded). Previous results[1] show the larger animal rhodopsin molecule to have about 100 H-bonded peptides, and just over 200 free peptides.

Some similarities between bacterial and animal rhodopsins have been pointed out by Osterhelt and Stoeckenius[5], including the use of a *cis*-retinaldehyde as a chromophore, the retinal to lysine Schiff-base linkage, and the effect of the protein on the retinal spectrum and on its chemical accessibility. Further, there is evidence that animal rhodopsin, like bacteriorhodopsin, has a quantity of α helix oriented predominantly normal to the membrane surface[7], and there is the suggestion that animal rhodopsin, like bacteriorhodopsin, may exist as aggregates of several monomers[8]. HX results point to another mild coincidence; though animal rhodopsin is considerably larger than bacteriorhodopsin, the extra length of polypeptide chain in animal rhodopsin has no regular secondary structure so that the structured regions of these two proteins are of roughly comparable size.

Considering possible relatedness of these proteins, it is interesting to examine the bacteriorhodopsin model for clues to the structure and perhaps the function of animal rhodopsin. We note the coincidence involving the previously suggested large channel in animal rhodopsin and the 20-Å 'channel' found in bacteriorhodopsin. While the channel in bacteriorhodopsin is filled in, with lipid[2], it has been suggested that animal rhodopsin maintains a functionally permeable channel by placing many of its extra amino acid residues within the channel with peptide groups exposed to maintain a polar milieu, therefore an aqueous filling[1]. The apparent structural similarities mentioned above suggest a supporting structural framework like that in bacteriorhodopsin for the maintenance of the channel in animal rhodopsin.

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Time-resolved resonance Raman spectroscopy of bacteriorhodopsin

BACTERIORHODOPSIN is found in purple patches on the cell membrane of halophilic bacteria[1]. Similar in structure to rhodopsin (but differing in function), it consists of a retinal–protein complex. Bacteriorhodopsin is a photosynthetic system which, when it absorbs light, passes through a cycle of several intermediates (Fig. 1) approximately 200 times per second, pumping protons from the inside of the bacterial cell to the outside. The resultant electrochemical gradient drives the phosphorylation of ADP to ATP (ref. 1). The intermediates have been identified by measuring their relatively broad structureless optical absorption spectra[2–4] as a function of time after a photolytic flash. The most precise structural information has come from resonance Raman spectra[5–8] taken with continuous wave (cw) optical excitation. These studies have

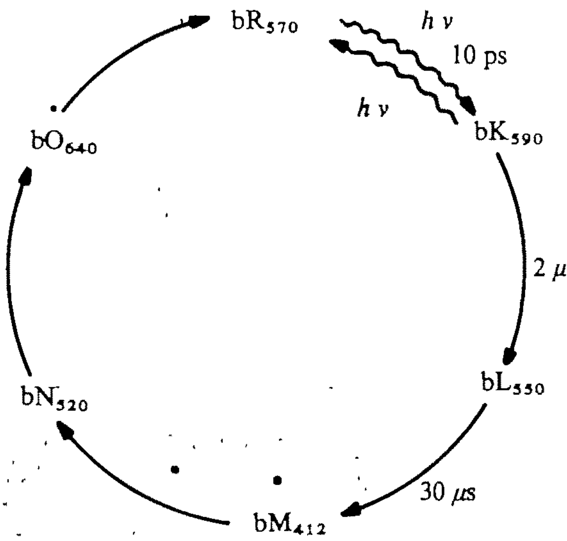

Fig. 1 Photochemical cycle of bacteriorhodopsin (see refs 2–4). Subscripts refer to the optical absorption maxima of each species (nm). Approximate half times are given for room temperature. One cycle is completed in several milliseconds.

provided information about the bR_{570} and bM_{412} intermediates only, since they have appreciable steady state concentrations at room temperature under constant illumination. Resonance Raman spectra of these species have also been obtained at liquid N_2 temperatures and in ether saturated solutions. These conditions, however, may not be representative of actual physiological conditions. We report here a time-resolved resonance Raman spectrum of an intermediate with a risetime of < 6 ns. Our data show a decrease in the C = C stretching frequency by 10–20 cm^{-1}. From the known inverse correlation between the C = C stretching frequency and the position of the absorption maxima[7] for a series of model retinal Schiff bases in various solvents[8], the observed C = C Raman band was assigned to the bK_{590} intermediate, which is known to have a risetime of 10 ps[4].

Time-resolved resonance Raman spectra of bacteriorhodopsin were obtained using a nitrogen pumped tunable dye laser (100-kW 5-ns pulses) at various excitation wavelengths near the maximum of the action spectrum of bacteriorhodopsin[10]. The gated detection system involved a PAR optical multichannel analyser coupled to low (15 cm^{-1}) and moderate (3 cm^{-1}) resolution monochromators using Corning cutoff filters to eliminate scattered radiation at the laser frequency.

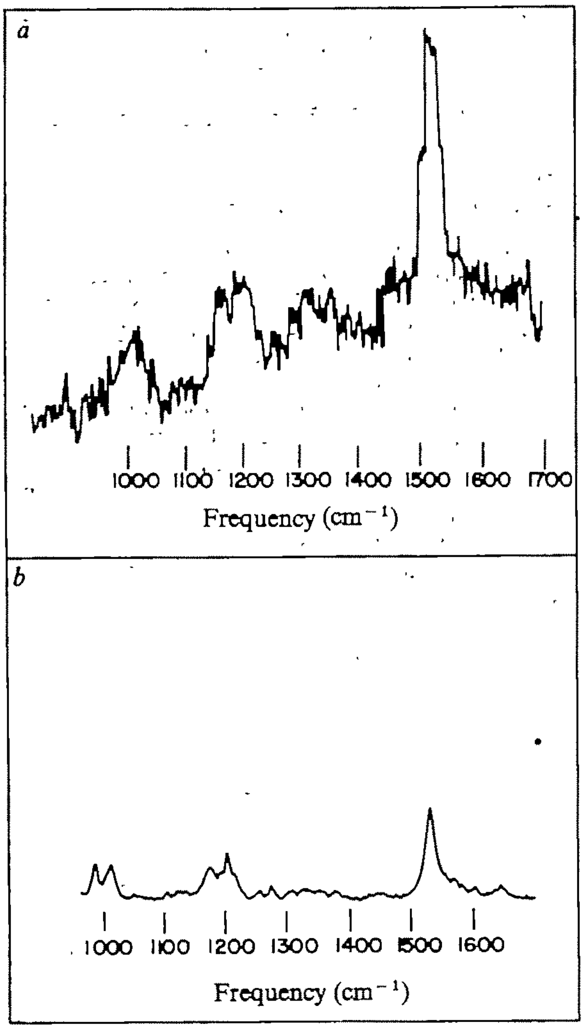

Fig. 2 *a*, Time-resolved (pulse-excited) and *b*, cw resonance Raman spectra of bacteriorhodopsin. The purple membrane fragments were extracted from *Halobacterium halobium* S-9 by the method of Becher and Cassim[11] and suspended in 4.3 M NaCl and 0.5 M $MgSO_4$ at concentrations of 60–100 μM. The time-resolved spectrum was obtained with 5480-Å excitation with a peak power of 100 kW (average power 10 mW) from a Molectron pulsed tunable dye laser. The cw spectrum was excited with 200 mW of cw 5145-Å excitation from a Spectra Physics Model 165 argon ion laser.

The low resolution spectrum with 5,480-Å pulsed excitation is shown in Fig. 2*a*. The prominent features are bands at 1,010, 1,210 and 1,530 cm^{-1}. The spectrum can be compared with a spectrum of bacteriorhodopsin taken with the continuous 5,145-Å line of an argon ion laser (Fig. 2*b*). This spectrum agrees in all respects with those previously reported in the literature[5–8] for bacteriorhodopsin. Considering the lower resolution of the pulse-excited spectrum, the close resemblance of these two spectra confirms that we observed the resonance Raman spectrum of bacteriorhodopsin with a pulsed ns source.

The 1,529 (±3) cm^{-1} band ($\nu_{C=C}$) in the pulse-excited spectrum was asymmetric in the low resolution experiment, so we recorded its band shape under higher resolution. The trace in Fig. 3*a* was obtained at an instrumental resolution of 3 cm^{-1} with the pulsed laser at 5,821 Å. The asymmetry evident in the low resolution scan is revealed to be a shoulder at ~ 10 cm^{-1} to lower energy than the main band which occurs at 1,528 cm^{-1}. The shoulder does not appear in any spectrum[5–8] taken with continuous excitation, regardless of excitation wavelength. The shape of the 1,530-cm^{-1} band measured with the 5,145-Å argon line is evident in Fig. 3*b*. Thus the shoulder seems to be due to the use of a pulsed excitation source.

We have ruled out the possibility that the shoulder is a trivial experimental artefact due to the presence of structure in the background (scattered laser light and fluorescence), by separately varying the wavelengths of excitation and position of the peak on the vidicon target. Both position and intensity with respect to the main peak were reproducible in these varying conditions. Comparison of spectra taken before and after prolonged irradiation by the pulsed laser showed no change in the relative position or intensity of the shoulder, eliminating a permanent photoproduct generated by the high intensity pulse as the species responsible for the shoulder.

We interpret this feature as arising from Raman scattering by the first intermediate in the photochemical cycle, bK_{590}. The repetition rate of the experiement (30 Hz) is much slower than the frequency of the photochemical cycle of bacteriorhodopsin (200 Hz) and thus prevents the formation of any appreciable steady-state concentration of the intermediates in the cycle. Optical absorption measurements have shown that the bK_{590} intermediate is generated in approximately 10 ps[4] and has a lifetime of 2 μs (ref. 2) at room temperature. Because the duration of the laser pulse is 5 ns, only bR_{570} and bK_{590} could contribute to the Raman spectrum. In addition, it has been shown[12] using optical absorption spectroscopy that the photoreaction $bR_{570} \rightarrow bK_{590}$ is photoreversible and that a photostationary state can be established during a 40-ns pulse with peak powers above several hundred kW. The relative concentration of bK_{590} and bR_{570} in this stationary state is 0.4. The intensity of the shoulder in our spectrum is approximately 0.4 that of the main peak at comparable (~ 100 kW) laser power and on a similar time scale.

The frequency difference between the peak and shoulder is consistent both in sign and magnitude with the correlations between optical absorption band maxima and vibrational frequencies reported for model retinal Schiff bases[8]. A 20-nm shift to the red of the absorption maximum of the chromophore would result in a 10–15 cm^{-1} decrease in the C = C stretching frequency, which is consistent with our data.

Our interpretation of this new feature is consistent with predictions from previous data on the lifetime and optical absorption spectrum of the bK_{590} species. We feel that we have demonstrated the utility of time-resolved resonance Raman spectroscopy in obtaining structural information about short-lived transients in photobiological cycles. Our experiments were carried out with acceptable, though not excellent signal-to-noise ratios, and we are currently redesigning the detection system for greater resolution and sensitivity. By using two pulsed lasers, one for photolysis and one as a probe beam we should be able to obtain the time-resolved resonance Raman spectra of other intermediates in the cycle. Furthermore,

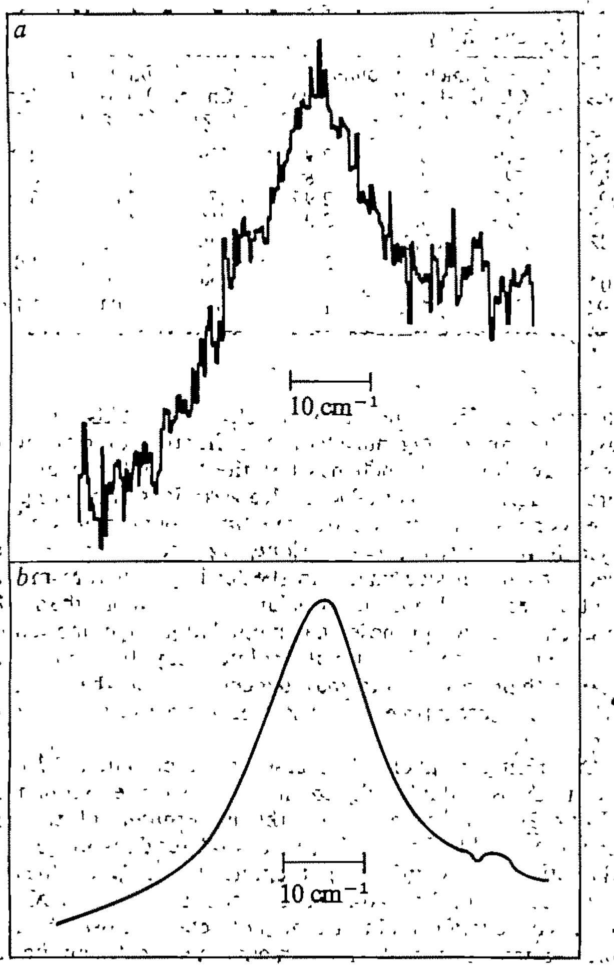

Fig. 3 *a*, Higher resolution of the pulse-excited (time-resolved) resonance Raman spectrum of the 1,530 cm^{-1} band of bacteriorhodopsin excited with 100 kW peak power of 5,821 Å from the pulsed dye laser. *b*, Expanded view of Fig. 2*b* to show the band shape of the 1,530 cm^{-1} peak in the resonance Raman spectrum of bacteriorhodopsin excited with a continuous source at 5,145 Å. The resolution is comparable with that in (*a*).

examination of the other bands in the time-resolved spectra should indicate at which point in the cycle deprotonation of the Schiff base occurs.

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Novel N-terminal protein blocking group identified as dimethylproline

THE N-terminal region of *Crithidia oncopelti* cytochrome *c*557 could not be characterised by conventional methods because of an apparently blocked amino function[1]. To study this feature the N-terminal tryptic peptide was isolated and 220 MHz proton magnetic resonance (PMR) spectroscopy was used to characterise this peptide and its hydrolysis products. We now report the identification of the novel blocking group as dimethylproline.

The tryptic peptide, known to have the structure X–Pro–$(CH_3)_3$Lys–Ala–Arg (ref. 1) was exposed to thermolysin cleavage to facilitate isolation of X-Pro–$(CH_3)_3$Lys. The trimethyllysine was then quantitatively removed with carboxypeptidase B. Amino acid analysis of X–Pro–$(CH_3)_3$Lys–Ala–Arg showed equimolar quantities of the four amino acids and no anomalous peaks. However, if the acid hydrolysate was separated by electrophoresis at *p*H 2.0 and the paper was sprayed with thymol blue (0.04% in 1 : 1 butanol–ethanol containing 0.01 M H_2SO_4) (ref. 2), an additional band was located which gave no colour with ninhydrin and had a mobility 0.9 that of proline. The indicator dye method depends on the buffering action of carboxyl groups; yellow (alkali) spots are seen on a red (acid) background.

The hydrolysis and electrophoresis was repeated on a preparative scale (3 µmol peptide), the band was eluted from the electrophoresis paper (Whatman 3MM), and the material was passed down a Sephadex G-10 column (100×1.5 cm) in 5% formic acid in order to remove material washed from the paper after electrophoresis. The PMR spectrum of this material is shown in Fig. 1 and the

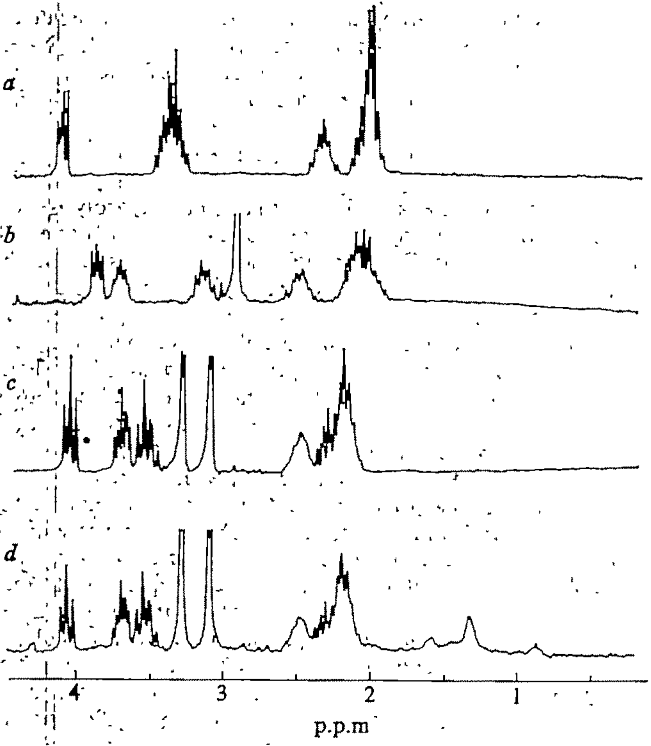

Fig. 1 PMR spectra of: *a*, proline; *b*, monomethylproline; *c*, dimethylproline standard, and *d*, X, isolated from cytochrome *c*557. Solutions were dried in a vacuum from 5% formic acid to remove traces of acetic acid and residues were taken up in 100% D_2O (0.3 ml). Concentrations were approximately 0.5 M for (*a*), (*b*) and (*c*) and 8 mM for (*d*). Spectra were recorded at 22 °C on a Varian HR-220 MHz, instrument modified for pulse Fourier transform operations with equipment supplied by Transform Technology Inc., Chemical shifts were measured relative to internal sodium 2,2-dimethyl-2-silapentane sulphonate.

Table 1 Parameters of PMR spectra shown in Fig. 1

Proton Assignment	Proline Chemical shift (Hz)	Proline Chemical shift (p.p.m.)	Proline Area	Monomethylproline Chemical shift (Hz)	Monomethylproline Chemical shift (p.p.m.)	Monomethylproline Area	Dimethylproline Chemical shift (Hz)	Dimethylproline Chemical shift (p.p.m.)	Dimethylproline Area	Unknown Chemical shift (Hz)	Unknown Chemical shift (p.p.m.)	Unknown Area
$\gamma_{1,2}$ β_2	443	2.01	2.92	456	2.07	2.82	479	2.18	2.9	479	2.18	2.95
β_1	511	2.32	0.99	542	2.46	1.02	543	2.47	0.83	534	2.43	0.98
Methyl-1	—	—	—	637	2.9	3.0	675	3.07	2.95	675	3.07	3.09
Methyl-2	—	—	—	—	—	—	716	3.25	3.0	716	3.25	3.17
δ_2	738	3.35	2.15	692	3.15	1.02	776	3.53	2.1	776	3.53	2.03
δ_1				812	3.69	1.09	809	3.68		809	3.68	
α	898	4.08	0.93	848	3.85	1.02	887	4.03	1.21	887	4.03	1.12

Areas were normalised to the nearest approximation to integral values.

spectral parameters are summarised in Table 1. Although the spectrum is markedly different from that of proline (Fig. 1*a*), the chemical shifts and areas of the peaks are consistent with a modified proline structure. The two sharp three-proton resonances occur in a region where methyl protons bound to nitrogen are found, suggesting that the material could be *N*,*N*-dimethylproline.

Dimethylproline was synthesised by alkylation of proline (50 mg) with methyl iodide (5 ml) in methanol (5 ml) under reflux. Alkaline *p*H was maintained by periodic addition of methanol saturated with NaOH. The synthetic material was isolated by passage down a Sephadex G-10 column (100×1.5 cm) in 20% acetic acid. It had a mobility on *p*H 2.0 electrophoresis identical to that of the natural material. The PMR spectrum is shown in Fig. 1*c* and summarised in Table 1, where it is compared with that of monomethylproline synthesised according to Bowman and Stroud[3].

It is clear that dimethylproline was present in the acid hydrolysate of the N-terminal peptide. The small amounts of material at 1.3 p.p.m. have been noted with several peptides purified by paper electrophoresis and this material is not completely removed by gel filtration. The evidence is consistent therefore with dimethylproline as the N-terminal blocking group of cytochrome *c*557 and the N-terminal region having the structure.

$(CH_3)_2$Pro–Pro–$(CH_3)_3$Lys–Ala–Arg

There remains the possibility that acid hydrolysis had modified the amino acid of the peptide to give dimethylproline. This possibility could not be investigated by enzymatic digestion as the $(CH_3)_2$Pro–Pro bond was resistant to a wide variety of exo- and endopeptidases. We have therefore examined the problem by comparing the PMR spectra of the natural peptides with that of synthetic dimethylproline. These results, which will be presented elsewhere, are consistent with the presence of dimethylproline in peptide linkage.

Dimethylproline is a novel structure in proteins. It was shown by Vickery[4] to be present in certain plants as the free amino acid and was named stachydrin. It presents an interesting problem in sequence determination and it is possible that it or other α-*N*-methylated amino acids may occur in other proteins. Because the amino group is no longer ionisable in these compounds they are invisible or unreactive using conventional N-terminal methods. The indicator dye method[2] affords a convenient means of visualisation, which was used for the identification of N-terminal amino acids by chemical methylation[2].

The N-terminal region of cytochrome *c*557 is ten residues longer than that of the vertebrate cytochromes *c*. It is possible that this 'tail' and similar 'tails' in plant cytochromes *c* (which are acetylated)[5] and fungal cytochromes *c* (in which the N terminus is free)[6] form relatively free-moving projections from the N-terminal α-helical region common to all cytochromes *c*. Perhaps the susceptibility to methylation of this region in cytochrome *c*557 is due to its detachment from the globular structure. This may also explain why, during purification, a fraction of cytochrome is always isolated which has lost the five N-terminal acids, presumably by a tryptic-like hydrolysis[1]. It is interesting that the lysine residues which are sometimes found methylated in cytochrome *c* sequences, residues 72 and 87 (ref. 7), are very prominent side chains in the tertiary structure models[8] and project out from the globular structure more than other lysines. Thus we propose that methylation of lysine, and in this cytochrome N-terminal proline, may be coincidental rather than specific and may depend only on the extent to which the susceptible group is free from the main protein structure.

A striking parallel to the case of cytochrome *c*577 exists in the myosin alkali light chains that have been sequenced[9]. Frank and Weeds note that the N terminus of the light chain A1 is blocked. This chain is 40 residues longer than the otherwise identical light chain A2; 25% of these 40 residues are proline and the N-terminal region is X–Pro–Lys. The peptide mobilities do not seem consistent with a methylated proline but an N-methylated acidic amino acid does seem a possibility. It would be interesting to re-investigate this region using methods for identifying hydrolysis products with free carboxyl groups.

The proline-rich nature of the N-terminal tail of residues is shared by the plant cytochromes. It is possible that all cytochromes at synthesis contain a proline-rich segment which might be necessary for setting the phase of the N-terminal helix perhaps for folding of the molecule or for the addition of the haem group. This segment may be retained in some and hydrolysed away in others depending on whether blocking of the N terminus had taken place.

Alternatively, a proline-rich segment may be conserved to minimise this very risk that the molecules should be digested by exopeptidases, since proline bonds are notably resistant to many proteases.

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matters arising

Middle Pleistocene stratigraphy in southern East Anglia

Rose, Allen and Hey[1] proposed a revised chronology for the Middle Pleistocene, based largely on the presence of a rubified sol said to be developed in a temperate climate, but no supporting chemical or palynological evidence was produced.

Extensive surveys conducted by ourselves and colleagues (Institute of Geological Sciences, Geological Survey Sheets 223, 240, 241) have shown that the following sequence of drift deposits is present.

The sequence below is the optimum development but field evidence shows elements of this succession consistently through the area. All these deposits (1–6) are therefore considered to show integral parts of one (Anglian) glacial cycle of events.

Interpretation	Lithological unit
Weathered profile or solifluction deposits	7, Heterogeneous brown flinty sandy clay
Late phase fluvioglacial deposits	6, Poorly sorted sands and gravels
Till, dominantly of the lodgement type	5, Chalky boulder clay variably decalcified at base
Flow till	4, Brown and grey mottled sandy clay with angular and rounded flints, involution structures
Proglacial lake deposits	3, Laminated sands, silts and clay
Proximal outwash braidplain deposits with local flow tills	2, Sands and gravels with localised developments of (4) and (5), including the basal chalky Maldon Till which may be of the lodgement type
Distal outwash deposits	1, Fine micaceous sands

A reddish colouration is developed at a number of horizons particularly where sandy clays have suffered remobilisation or eluviation has taken place from the lowest clay bed in the sequence. The eluviation process was apparently accompanied by dissolution of limestone material from the permeable beds where the ground-water was not lime-saturated. It seems unlikely that each of these horizons could be regarded as indicating a warmer period.

The thin and localised development of the Anglian fluvioglacial deposits suggested by Rose, Allen and Hey[1] is questionable for an area near the margin of an active ice-sheet. There is evidence for the former presence of an active ice sheet in the Thames Estuary[2] area which may have influenced the outwash drainage of the main East Anglian ice and thus account for the deduced north-eastward flow[1] of the lower gravels.

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[1] Rose, J., Allen, P. & Hey, R. W. *Nature* 263, 492–494 (1976).

[2] Lake, R. D., Ellison, R. A., Hollyer, S. E. & Simmons, M. *Rep. Inst. geol. Sci. U.K.* (in the press).

Rose, Allen and Hey reply—In response to Lake *et al.*[1] we wish to clarify the following points:

(1) Recognition of the temperate palaeosol is based not only on red colouration but on clay enrichment, iron enrichment, and clay skins on the upper sides of pebbles. These properties are considered characteristic of the illuvial horizon of a *sol lessivé* formed over a long period in a humid warm temperate climate. We are aware that iron stained horizons are common at many levels in the lower sand and gravel unit, but unlike the palaeosols horizon, these levels are not accompanied by a significant clay enrichment or clay skin development, and their colour does not reach levels of redness as high as 2.5 YR or 10 R. These iron-stained horizons are not continuous and are not developed at any particular level, but are specifically associated with sedimentary structures along which ground water drainage has been localised. The properties of the rubified *sol lessivé* are developed independently of the sedimentary structures and the original particle size characteristics.

(2) Lake *et al.* state that "sandy clays have suffered remobilisation, or eluviation has taken place from the lower clay bed in the sequence", to account for the "reddish colouration". If these processes were responsible, however, reddish colouration should be continuous along the interface of the sands and gravels and the overlying till. This is not the case. At certain localities the palaeosols horizon is cut-out by till. At other localities it is overlain by the upper sand and gravel unit and is thus not associated with a 'clay bed'. As the palaeosols horizon is cut-out by glacial and glacifluvial erosion, and as blocks of the palaeosol form erratics in the overlying till, rubification has occurred before ice invaded the locality.

(3) The observation that "the eluvial process was apparently accompanied by dissolution of limestone material from the permeable beds where the groundwater was not saturated" is incorrect, as fresh, hard limestone pebbles have been observed in all parts of the lower sand and gravel unit.

(4) An arctic structure soil is superimposed on the *sol lessivé*. This consists of an horizon of involutions, ice wedge casts, and sand wedges and is associated with coversand, loess, and wind polished stones. This indicates a long period of sub-aerial weathering in addition to the *sol lessivé*, and also demonstrates that the sequence of deposits were not formed in a single "glacial cycle of events".

(5) The revision of the stratigraphy is not solely based on the recognition of the palaeosols horizon. The glacifluvial sands and gravels above the palaeosols can be distinguished from the underlying periglacial river deposits on the basis of particle size, stone content, primary sedimentary structures, secondary sedimentary structures, palaeocurrent directions[3], and heavy mineral assemblage (J. A. Catt, personal communication). The heavy minerals in the lower sand and gravel unit show a restricted residual suite, whereas the range

of heavy minerals in the upper sand and gravel unit is greater and many readily-weatherable minerals are included. This suggests that the heavy minerals in the upper sand and gravel unit have been enriched from a new source, such as would be associated with a glacial event. Also the frequency of volcanic rocks, quartz, and quartzite is much greater in the lower sand and gravel unit than in the till. Thus it is impossible to argue that these materials could have been derived either from the till or from the ice which deposited the till.

(6) We are interested to know on what basis active ice in the Thames would affect outwash drainage as far away as Bury St. Edmunds.

(7) Anglian glacifluvial deposits are restricted because most of the meltwater beneath the ice was concentrated in 'tunnel valleys' related to a low sea level[3]. The relative scarcity of glacifluvial deposits in an ice-marginal location is not peculiar to southern East Anglia. It is also characteristic of the southern margin of the Devensian ice sheet.

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The cause of Forbush's 20-year wave

Levy[1] has suggested an explanation of Forbush's[2] 20-yr wave in the galactic cosmic-ray diurnal anisotropy. Levy states he knows of no other way to account for the variation. This note discusses a previously known alternative mechanism and provides a calculation for the thickness of the heliosheath.

Any mechanism to explain Forbush's 20-yr wave must use the 20-yr oscillatory solar cycle vector dipole field (B_{SD}). To obtain a sinusoidal wave from this vector, it is necessary to have a mechanism which basically takes the product of B_{SD} with a stationary vector. Levy's mechanism utilises the azimuthal twisting of the interplanetary magnetic field (IMF) lines to obtain an enhanced radial $\nabla \times B$ drift between the positive and negative field of the extended solar dipole. The azimuthal twisting of the field as a result of the solar rotation is important because the perpendicular drift in a radial field would only result in an azimuthal drift of the cosmic rays[3] with no outward 20-yr wave. Thus Levy's mechanism takes the product between B_{SD} and the angular velocity vector of the Sun to obtain an effect.

Schatten and Wilcox[4], however, take the product between B_{SD} and the nearby galactic magnetic field (GMF) vector to obtain their effect. In this model, cosmic rays either gain access to the heliosphere at high (or low) heliographic latitude depending on whether the extended IMF connects to (or is closed from) the GMF. This causes a 20-yr streaming of cosmic rays away from (or toward) the Sun along the IMF of the type Forbush observes, and Duggal and Pomerantz[5] confirm.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

It is interesting that this latter view, if correct, enables a calculation of the thickness of the heliosheath. The cosmic rays producing the Forbush effect have an energy of around 25 GeV (refs 1 and 2). Thus their rigidity is $R\ \mathrm{V} \doteq 300\, H\rho$ (H in gauss, ρ in cm).

The heliosheath fields are most effective at filtering cosmic rays with gyroradii comparable to the heliosheath thickness $\rho \sim d$. An extension of the Archimedean spiral IMF suggests H may be as low as 6×10^{-7} G whereas the galactic magnetic field is nearer 10^{-6} G (ref. 6). Equating p to d in the above equation, we find for d, a value of 1.4×10^{14} cm $\sim$ 10 a.u. This is in fact approximately the value suggested for the heliosheath thickness[7]. Undoubtedly Pioneer missions to the outer planets may add to our knowledge about what is happening in this interesting region of space.

I thank M. A. Pomerantz for valuable discussions. This research was supported by the Atmospheric Research Section of the National Science Foundation.

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Levy replies—The suggestion put forward by Schatten and Wilcox[1], that the solar-magnetic-cycle wave in the diurnal variation of energetic cosmic rays results from alternating connection and disconnection of the interplanetary and galactic magnetic fields, is an interesting one that deserves quantitative discussion. Although this is not the place to discuss the idea in detail, one remark does seem in order.

As I understand Schatten's present calculation, particles with cyclotron radii that are small compared with the characteristic length scales in the magnetic-field reconnection region will efficiently follow the field lines and thus will show the largest effect of field line topology. Particles with increasing energy, and thus increasing cyclotron radii, will be less inhibited in their motion by the details of the filed-line topology in the heliosheath reconnection region. Thus it seems to be a natural consequence of Schatten's and Wilcox's proposal, in its present form, that the amplitude of the variable anisotropy is a monotonically decreasing function of particle energy. The existing, but sparse, information on this point[2] suggests that this is not the case. The amplitude of the varying anisotropy seems to increase with energy in the range below 20–30 GeV and to decrease at larger energies. This behaviour is consistent with that predicted as a result of particle drifts[3] in a large-scale coherent interplanetary magnetic field[4].

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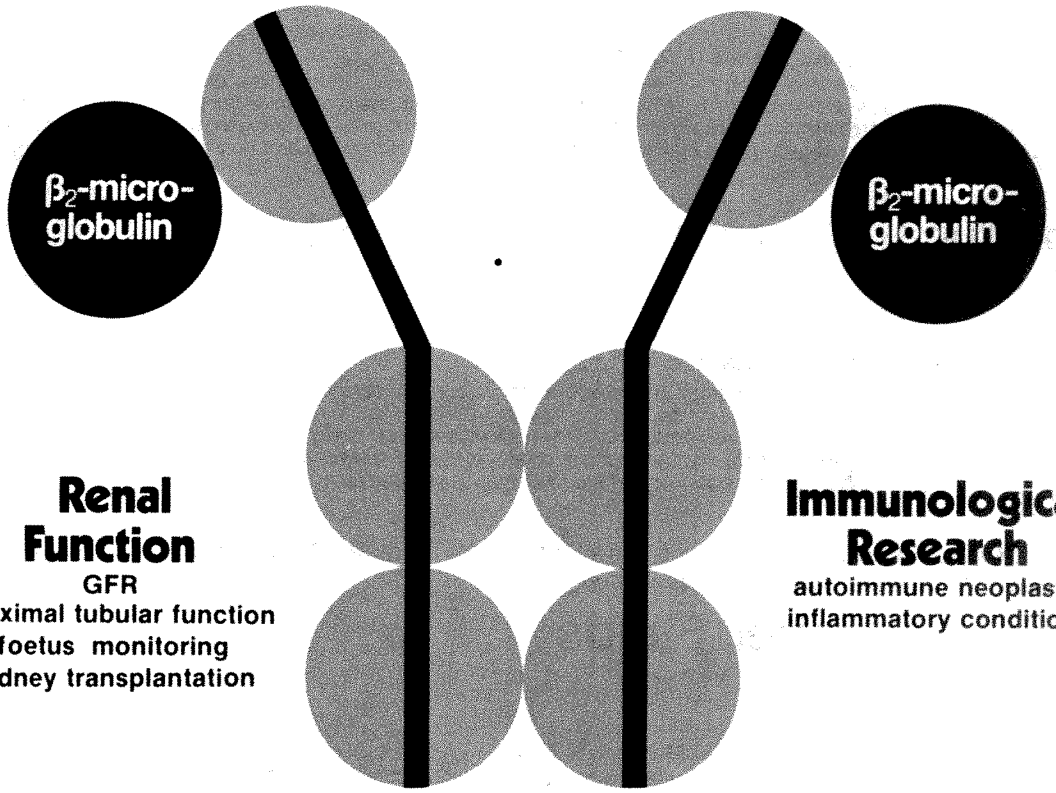

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This book is the product of an inter-disciplinary seminar arranged to encourage more fruitful cross-fertilization between social anthropologists, psychiatrists and psychoanalysts. Believing that its chosen theme is of central importance to both disciplines, this symposium addresses the problem of interpreting the emotional as well as the cognitive meaning of symbols. The papers span a rich diversity of cultural and historical settings and take the connection between symbolism and sentiment as their unifying force.

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edited by A. N. Davison

March/April 1977, xiv + 346pp., £14.00/$27.35 0.12.206650.2

In recent years, with the delineation of the broad principles of intermediary metabolism of the brain and the identification of specific brain constituents, it has been possible to begin the important task of correlating structure and function of the nervous system with its biochemistry. In this collection of studies, neurobiologists from widely different disciplines survey recent research on the biochemical basis of normal brain structure and function, with the emphasis on an interdisciplinary viewpoint. The book falls into three sections: the developing brain; chemical-structural correlations; and the biochemistry of behaviour.

Proceedings of a Symposium Organised by the London Mathematical Society with the Support of the Science Research Council and the Royal Society

Algebraic Number Fields

(L-functions and Galois properties)

edited by A. Frohlich

February/March 1977, x + 704pp., £16.00/$31.25 0.12.268960.7

This volume is the outcome of a research symposium in algebraic number theory, held in September 1975 at the University of Durham, England. Almost all the lectures given are recorded here, and in many cases they have been expanded and new, relevant material added. The specific topic of the symposium was 'L-functions and Galois properties of algebraic number fields' — an area which is currently undergoing exciting, fundamental developments, and the volume reports on these. It contains contributions of an introductory nature, surveys of recent work which has appeared in a variety of technical papers, and also a number of original papers whose contents have not previously been published.

Proceedings of the Second Conference on the Mathematics of Finite Elements and Applications

The Mathematics of Finite Elements and Applications II — MAFELAP 1975

edited by J. R. Whiteman

March/April 1977, xiv + 573pp., £21.00/$41.00 0.12.747252.5

This is the proceedings of the 2nd Conference on the Mathematics of Finite Elements and Applications, held at Brunel University in April 1975. The purpose of the Conference was to bring together mathematicians and engineers whose common interest was finite element methods. The resulting book consists of eleven invited papers and thirty-two short contributed papers, with the invited papers defining the state of the art of finite elements and the contributed papers describing current research. The papers fall into three categories: the mathematics of finite elements; applications; and computational techniques. In this way the book both breaks new ground and is a reference work.

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reviews

Adaptation to marine life

R. P. Dales

Adaptation to Environment: Essays on the Physiology of Marine Animals. Edited by R. C. Newell, Pp. 539. (Butterworths: London and Boston, Massachusetts, November 1976.) £25.

THIS book comprises nine separate reviews: Adaptations to intertidal life by the Editor himself; Settlement responses in marine organisms by Professor Crisp; Primitive respiratory mechanisms by C. P. Mangum, Physiological adaptation to life in estuaries by A. P. M. Lockwood; Rhythms, behaviour and reproduction in marine animals by Professor Naylor; Vision in pelagic animals by J. K. Bowmaker; and three chapters by P. W. Hochachka and G. N. Somero: Biochemical adaptations to pressure; Enzyme and metabolic adaptations to low oxygen, and Biochemical adaptations to temperature.

Nine chapters within the compass of over 500 pages has provided each author with sufficient space to review in some detail the current concepts and knowledge in their own field. Any of these reviews might have appeared in either of the volumes of reviews in marine biology currently being published by Academic Press or Allen and Unwin. Such a volume as this may be justified by the amount of work being produced or by the individual contributions making a coherent whole.

It is six years since Newell's book on the *Biology of Intertidal Animals* appeared and so much interesting research has been done since then that in many ways I regret that the field was not restricted to environmental adaptations of intertidal animals. This is not to welcome Bowmaker's contribution on vision for, although this chapter is perhaps the most isolated in subject matter from the others, a review of this subject was much needed. And, although the literature is less extensive than that reviewed elsewhere in the book, the most significant recent papers are not readily available. It is also a more difficult subject for most students, so that it is good to have this literature authoritatively assessed.

This is an invaluable book to all those interested in the adaptation of organisms to their environment. Much of the contents are familiar, but the incorporation of recent work and subsequent discussion is valuable for students and teachers trying to keep up-to-date in this field.

Thus Crisp's review of 1974 is amplified, and Naylor's review of rhythmic behaviour is to be welcomed because it brings together various aspects of the subject such as circadian rhythms, vertical migration and reproductive cycles. Mangum's review also pulls together the many recent contributions in the field of respiratory pigment function. This last is particularly interesting for the comparison of intra- and extracellular respiratory proteins and for her ideas on the evolutionary significance of red cells in invertebrates. Lockwood's chapter on the physiology of estuarine organisms is useful too, not only because it is his own account but because other recent reviews and treatises have been more ecological than physiological in approach.

The three chapters by Hochachka and Somero also bring up-to-date the subjects discussed in their recent book on *Strategies* (Saunders: Philadelphia, 1973), in *Comp. Biochem. Physiol.* and in the Society for Experimental Biology Symposium on *Pressure*, edited by Sleigh and Macdonald. Most of the chapter on low O_2 adaptations will be familiar to those who know *Strategies* but the more recent work done both by Hochachka's own group and by de Zwaan's group in Utrecht on bivalves, is included here.

A most useful series of essays on physiological and biochemical adaptation, well printed and illustrated, a book no zoological library can afford to be without. It is a pity that rising costs puts such a book beyond the reach of most individuals. ☐

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Ion implantation

Ion Implantation of Semiconductors. By G. Carter and W. A. Grant. Pp. viii+214. (Arnold: London, 1976) Boards £9.50; paper £5.95.

ION IMPLANTATION was for a number of years a specialist subject studied only by research scientists and engineers. The past five years has seen the increased acceptance of the technique in the semiconductor device manufacturing industry and a growing awareness of its potential in other areas. It is not surprising, therefore, that a number of educational establishments have introduced courses on the subject. It is clearly intended that this book should provide a student text for such courses. For this purpose the book is well suited.

It guides the reader in a logical manner through the physics of ion implantation. Starting with very elementary concepts, all aspects of the subject are explained. There is an unfortunate over simplification of the theory of ion range calculations. It has been assumed, without apparent justification, that implanted profiles are symmetrical, whereas the physics of observed asymmetric profiles is well understood.

The greatest shortcoming of the book is in the treatment of the electrical effects of ion implantation in semiconductors. Most of the book could be applied equally well to metals and insulators. As it is the electrical effects in semiconductors that have made them of special technological interest it was very disappointing to find that the discussion on this aspect did not merit a chapter on its own but was included in a rather abbreviated devices chapter. This has meant that important topics such as electrical measurements during annealing have been curtailed and the electrical properties of compound semiconductors virtually omitted. It has also meant that some important device application areas such as threshold changing in MOS transistors were not included.

In summary, the book is likely to be useful as a general introduction to the subject but should not be regarded as being specific to semiconductor applications.

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Chemical carcinogens

Chemical Carcinogens. (ACS Monograph 173.) By C. E. Searle. Pp. xxvii + 788. (American Chemical Society: Washington, DC, 1976.) $67.50.

THAT most cancer in man is caused by chemicals is now accepted, although the first demonstration of cancer produced by a pure chemical compound was only published in 1930 as a postscript to a paper by E. L. Kennaway. Before that there was evidence that soot, coal tar, pitch, and some oils caused cancer in man; and soon after Kennaway's successful experiment the widely distributed carcinogen, benzopyrene, was isolated from coal tar and synthesised. These experiments opened the gates to a flood of research in chemical carcinogenesis. Most cancer in man is caused by chemical substances in the environment and a sure way of preventing cancer is to identify these carcinogens and remove them. Such removal is not always feasible in practice. The most ubiquitous cause of cancer in Britain is tobacco but it seems impossible to prevent smoking. The active chemical carcinogen or carcinogens in tobacco smoke are still not known and attempts to make 'safer' cigarettes are hampered by the lack of knowledge.

The existing knowledge of chemical carcinogens and the research in the problems are so vast that it is difficult for individuals or even teams of workers to cover the subject. The volume under review is an admirable and much needed attempt to present the available facts and some of the theories. The main gap in the book is that inorganic carcinogens, except asbestos, are not considered.

The editor points out that this is not the first effort to list a catalogue of chemical carcinogens. The US Department of Health, Education and Welfare has published a series entitled *Survey of Compounds Which Have Been Tested for Carcinogenic Activity,* some of which were edited by Hartwell and Shubik The first of the series appeared in 1951 and the last, which is generally available, includes data published in 1971. The International Agency for Research on Cancer in Lyon has published twelve monographs on the *Evaluation of the Carcinogenic Risk to Man.* These monographs are invaluable to research workers and administrators concerned with cancer problems. In very few cases, however, is it possible to evaluate the potential carcinogenic hazard to man of pure chemical compounds.

In addition to the preface written by the editor, which is an excellent review of the present position of the subject, there are 16 chapters written by 23 experts in their fields. There is less repetition than would be expected with a book written by so many authors. The chapters on "Soots, tars and oils" by Kipling, on "Laboratory hazards" by George and Searle and on "The Bracken Carcinogen" by I. A. Evans and "Asbestos" by Wagner, are each about 10 pages. The chapter on "*N*-Nitroso Compounds" by Magee, Montesano and Preussmann is an authoritative review of the subject covering 125 pages. In the case of this and other articles it would be of value and interest to know when they were written.

There are also comprehensive reviews on endocrine aspects, alkylating agents, polynuclear aromatic carcinogens, environmental respiratory carcinogenesis, aromatic amines, carcinogens in plants and microorganisms, and metabolism of chemical carcinogens.

The book is well produced, easy to handle considering its size, and provides an excellent overview and reference work, although it is already out of date in some minor matters.

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Perception handbook

Handbook of Perception. Vol 7: Language and Speech. Edited by E. C. Carterette and M. P. Friedman. Pp. xviii + 501. (Academic: New York and London, 1976.) £16.25.

A SUCCESSFUL handbook has the opposite fate to a 'classic': it is consulted, but not quoted. It is used to settle disputes, to aid memory, and to fill in inevitable gaps in knowledge. To these ends it must be well organised, comprehensive, and authoritative. In psychology, the standard was set by S. S. Stevens's *Handbook of Experimental Psychology* which was first published in 1951. Carterette and Friedman are engaged in the publication of a more ambitious work, a ten-volume handbook intended "for all in the arts or sciences . . . who are interested in human perception". The first six volumes are concerned with fundamentals, and the present volume is the first to be devoted to a wider consideration of the contents of perception rather than particular processes or modalities.

There are fourteen chapters arranged in four parts: fundamental aspects of language and speech; coding, perception, and hearing; disorders of language and speech; and trends in psychological tests of linguistic theory. These headings are not auspicious and certainly do not reflect a natural organisation of the field. The assignment of topics to them turns out to be fairly arbitrary, and the problem of finding one's way through the book is further aggravated by a considerable degree of overlap between the contents of the chapters Accounts of the production of speech crop up in three different places, varying considerably in detail, and there are numerous cases of brief descriptions of the same work occurring in more than one chapter—for example, the subjective location of clicks, Katz's theory of semantics, and Winograd's program for understanding natural language.

The book ranges reasonably comprehensively over the major topics in the area, and includes useful chapters on speech perception (C. Darwin), the syntactic analysis of sentences (J. M. Carroll and T. G. Bever), the research methods of psycholinguistics (G. Olson and H. Clark), non-verbal communication (J. Laver), and aphasia (H. Goodglass and N. Geschwind). Somewhat surprisingly, it also has chapters on language teaching and language learning, disorders in speech production, and the general development of language in children. Yet, a number of topics very relevant to perception do not have chapters devoted to them—for example, intonation and tone of voice, the psychology of reading, and computer programs for understanding speech. At a more technical level, the reader will look in vain for an account of linear prediction theory, augmented transition networks, the perception of illocutionary force, and procedural semantics. It is probably no coincidence that each of these topics reflects an increasing concern with psychological processes in real time rather than with the static structures of linguistic theory. Ironically, the chapter that best captures this growing preoccupation, MacNeilage and Ladefoged's excellent discussion of speech production, is concerned with perception in only a tangential way.

In summary, the compilers have solicited a number of authoritative articles from acknowledged experts, but they have failed to cover the field comprehensively and to edit the materials to the best advantage. They have produced a superior symposium rather than an indispensable handbook.

P. N. Johnson-Laird

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Problems of memory representation

Processes of Animal Memory. Edited by D. L. Medin, W. A. Roberts and R. T. Davies. Pp. xi+267. (Hillsdale, Lawrence Erlbaum Associates: New Jersey, 1976. Distributed by the Halsted Press Division of Wiley: New York and London, October 1976.) $19.00; £11.50.

THIS book is useful because it collates a set of papers by authors who have all worked closely together in the past on problems of memory representation. It demonstrates that the problems of inference of the existence and nature of mental representations in animals are, indeed, formidable, but that with ingenuity much can be said about such possibilities, and that perhaps we can no longer talk sensibly about learning theory if we omit them. It also shows that the questions raised when proper investigations are made are far more interesting than those with which psychologists have fobbed off laymen and bored each other for so long.

The first paper by Ruggiero and Flagg directly takes up these issues. It reviews neglected early work, and describes interesting new work by Medin and Davis, among others, which shows that monkeys find some patterns easier to learn than others. This fact seems trite until the data are considered in terms of the regularities of patterns which are particularly striking to animals. It is not simply that such regularities may reflect the organisation of perceptual analyser systems as postulated by Sutherland, Mackintosh, *et al.* The regularities may also usefully be described in terms of the principles of encoding economy which have been so useful in extending the application of information theory to human pattern recognition. We may talk, though still loosely, of an animal making "the best sense" of a pattern, by selectively remembering its critical rather than incidental characteristics.

The application of paradigms and models conventional in the study of human short-term memory to animal work is a major theme of this symposium. A paper by Davis and Fitts shows that the modifications of paradigms required is not as great as had previously been thought. Although the empirical data they present are as yet too scanty for theoretical treatment, they have already shown that provocative similarities and differences in obtained data do not demand separate theoretical constructs for humans and monkeys.

A charming paper by Bolles revives the study of 'insight' in animal learning which has always been a standing challenge to behaviourists, and which is the current preoccupation of modern systematic theorists such as Levine. There is, perhaps, too much emphasis on the "Garcia effect", or "stomach memory", that is, the tendency of even lower mammals, such as rats, to avoid specific foods when they have been poisoned (non-fatally!) up to an hour after eating them. Demonstrations that rats will avoid eating in one place rather than another under similar circumstances are interesting. But a further demonstration by Riley that animals poisoned after drinking water show subsequent aversion to novel solutions such as saccharine (control data are not quoted) are less interesting than the author supposes. Rats always tend to avoid novel foodstuffs, and show a cautious empirical approach, eating very little until no uncomfortable consequences have ensued on several occasions. Although rat-catchers may feel their darkest suspicions of rodent cunning have been confirmed, it is still too early to take these as demonstrations of the understanding of 'causal relationships' by lower animals, as Bolles would wish us to do. He is nevertheless quite right to point out that it is time to seriously consider whether the rigid tenets of stimulus–response learning do not require further testing in this regard. The perception of "rules" and "regularities", and the perception of "causality" shade into each other in ways uncomfortable for older theorists.

A concluding paper by Whitlow attempts a direct reinterpretation of the most elementary S-R learning theory, classical conditioning theory, in terms of temporal organisations of events ("episodic memory theory") now current in the literature on human memory. The conceptual failures involved are illuminating. Perhaps as much for the human as for the animal work. **Patrick Rabbitt**

Patrick Rabbitt is Lecturer in Psychology at the University of Oxford, UK.

Whither goest palaeoecology?

Structure and Classification of Paleocommunities. Edited by R. W. Scott and R. R. West. (Wiley: New York and London, 1976.) £18; $30.

THIS child is the offspring of 15 authors and was born two years after an American Paleontological Society Symposium held at Miami Beach in 1974. The creative impulse was triggered by the question "Whither goest palaeoecology?". The book consists of 11 chapters of which the first four are basically reviews of community concepts in palaeoecology (Kauffman and Scott), two chapters on classification in terms of trophic levels (Scott) or environments (Parker), and a comparison of living and dead assemblages (Macdonald). The remaining seven chapters are more specific studies related only by having a palaeoecological bias. They range from living communities of the S. Californian shelf (Stanton), through Mexican and Californian living and recently dead molluscs and foraminifera (Warme, A. A. Ekdale, S. F. Ekdale and C. H. Peterson), Pleistocene ostracodes from Utah (Lister), Eocene nonmarine molluscs from Wyoming and Colorado (Hanley), seven lingulid communities of different ages (West), Pennsylvanian ostracodes from Kansas (Brondos and Kaesler), to Mississippian communities from Georgia (Broadhead).

Understandably definitions of communities differ. "A paleocommunity is the suite of preservable taxa comprising a community (pvi)"; as for communities they can be regarded as "a unique congregation of diverse organisms having a unique structure based on organism interactions . . ." (p18); "recurrent groups of numerically co-occurring species" (p143); and (p88) "a group of species which are often found living together".

Most of the authors avoided answering the original question of where the subject was going and preferred to explain where they and others had been. And since vertebrates, plants, trace fossils and substantial parts of the invertebrate palaeoecological record were, in effect, not touched on (and in fairness to the authors nor could they have been within the compass of a slim volume) it is not surprising that the product is a ragged patchwork of individual interests. The frequently overlapping references and interests are predominantly American. A *much* more interesting project would have been to pose the same question, quite separately, to another group of 15 palaeoecologists in the States, and comparable groups in say, Canada, Britain, France, Germany and Russia, and then compare the products.

The blurb claims that the book serves as a fascinating historical overview of the significant advances in community ecology and palaeoecology from the 1950s to the present day, and is an unprecedented summary of current knowledge. The publishers have prudently set the price at £18.00. **G. Y. Craig**

G. Y. Craig is Professor of Geology at the University of Edinburgh, UK.

Anti-bacterial therapy

The Battle Against Bacteria: A Fresh Look. By P. Baldry. Pp. 179. (Cambridge University: Cambridge, London, New York and Melbourne, 1976). £4.50.

ONLY six of the 15 groups of anti-bacterial drugs now in wide use were available 20 years ago. Since then, however, the spectre of transferable drug resistance has become the constant adversary of microbiologist and clinician alike. These dramatic changes are emphasised by even a superficial comparison of the two editions of Dr Peter Baldry's history of anti-bacterial therapy. The first edition, although published in 1965, took the story only up to the late 1950s and occupied a compact 100 pages. Inevitably its successor is over 70% longer. It describes the evolution of a field which is a fascinating meeting ground of microbiology, clinical medicine, biochemistry, pharmacology and molecular biology.

The book is least successful when summarising the history of bacteriology as distinct from therapy. Thus Leeuwenhoek did not see the 'animalcules' which cause disease; he saw only saprophytes and normal commensals. His illustrious predecessor Robert Hooke is not mentioned, even though an engraving from Hooke's *Micrographia* is included (without attribution). Joseph Lister's pioneering bacteriological work—which included isolating the first pure bacterial cultures —also goes unnoticed, as does his even more relevant observation of 1871 on the antibiotic action of *Penicillium* cultures. Moreover, Lister was not Professor of Surgery at Edinburgh but in Glasgow at the time of his original discovery of antisepsis. The romantic legend of the martyrdom of Semmelweiss also needs correction. This tragic pioneer of hand disinfection in obstetrics in fact suffered from chronic paranoia. After increasing insanity necessitated his commital to a mental hospital his death from presumed streptococcal septicaemia—following a hand injury—was supreme irony. For this was the infection he had prevented in thousands of women during childbirth.

The author is on much firmer ground with his main theme—the discoveries of anti-bacterial drugs. There are only two major disappointments here—apart from the lack of any references: the story of the British work on sulphonamides (culminating in M&B 693) is omitted, and the central chapter on Fleming's original observations of 1928 remains unchanged from the first edition. There is no mention of the fascinating revelations published in 1970 by Fleming's colleague, Ronald Hare. In his book *The Birth of Penicillin*, Hare showed that almost every detail of the familiar story is erroneous. Nevertheless, Dr Baldry's book is a good introduction to an enthralling subject. It should attract a wide audience.

Sydney Selwyn

Sydney Selwyn is Reader in Medical Microbiology at Westminster Medical School, London, UK.

Noradrenaline and adrenergic nerves

Peripheral Sympathetic Neurotransmission. By W. De. Potter. Pp. xv+161. (Arscia Uitgaven, NV: Brussels, 1976.)

THIS book is a strongly personal account of the longstanding research interests of the author and his laboratory. Since he is an acknowledged leader in his field, it is predictably a good account of the biochemical aspects of the synthesis, storage and release of noradrenaline from adrenergic nerves. Factors affecting the storage and release, such as electrical stimulation, and α- and β-receptor-blocking agents, are also described. The relationship between the different noradrenaline-containing particles is discussed. There is a clear and informative diagram at the end of the book which summarises the author's views of the origin and fate of noradrenaline storage particles. The sections on methods are especially good and should be most useful to those engaged in this area of research. The bibliography is fairly extensive (270 references) although it stops at 1973.

My one reservation is related to the title. In my opinion, this title implies a general coverage of all aspects of transmission including information on neuroeffector geometry, the electrophysiology of transmission, and effector cells including 'receptor' characteristics. These topics are not discussed. There is also little account of transmitter inactivation mechanisms. I have, however, no hesitation in recommending this book for its lucid description of storage and release of noradrenaline from sympathetic nerves. It would make a useful addition to the library of anyone working in the field of neurotransmission. **G. Burnstock**

G. Burnstock is Head of the Department of Anatomy and Embryology at University College, London, UK.

Electric fields and isolated atoms

Atoms and Molecules in Electric Fields. By N. Ryde. Pp. 455. (Almqvist and Wiksell International: Stockholm, 1976.)

THE effect of external electric fields on the spectra of isolated atoms and simple molecules has been investigated since early this century. In particular the splitting and shifts in spectral lines observed as the external field is increased, resulting from the perturbation of otherwise spatially degenerate levels, have been important in the development of quantum theory. These 'Stark effect' measurements continue to provide important information on oscillator strengths and the assignment of spectra. Increasingly though, interest has shifted to the application of these effects, for example, in astrophysics or plasma studies where the broadening or line shift can be related to the electron density and temperature. There is thus a need, identified by the author, for a critical compilation of the large amount of data amassed in the past forty years for use in these new areas.

The most substantial and useful section of this book forming about 50% of the whole is just such a compilation of the experimental or calculated electric field properties of some fifty atoms and atomic ions, much of this work dating from the 1920–1930s. Spectral assignments are discussed and plots of line displacement with field strength are recorded here. A large part of the remainder of the book is concerned with the quantum theory of these processes. This section begins with a detailed account of elementary perturbation theory and then reviews the application and development of these techniques in a wide range of atomic and molecular situations.

The other chapters in the book dealing with experimental matters and molecular species are rather limited in scope and will be of only limited interest to the type of reader for whom this book is intended. The use of the phrase 'canal ways' throughout the book makes a curious impression. There is no index.

This book will be a useful reference for those using the atomic 'Stark effect'. **Malcolm Fluendy**

Malcolm Fluendy is Lecturer in the Department of Chemistry, University of Edinburgh, UK.

nature February 24, 1977

Human rights and the scientist

'SCHOLARS of all kinds continue to be oppressed and persecuted in many parts of our contemporary world, and concerted attempts to impede their enquiries and suppress their conclusions are as common today as at any time in the past'. So begins a newly published report *Scholarly Freedom and Human Rights* (Barry Rose Publishers Ltd, £2.25) by a study group of the Council for Science and Society in collaboration with the British Institute for Human Rights. No documentation is given for the statement; indeed, the group has deliberately avoided quoting examples, but anyone with a mite of political awareness will know exactly what they are talking about and also that oppression is not restricted to any particular part of the world.

The central theses of the report are two: that there is a substantial body of international human rights law which should be adequate to cope with the sort of violations that are depressingly familiar; and that scientists, depending so much on communication, are peculiarly prone to what may seem to others to be minor constraints or irritations. Isolate an accountant from the literature and from his colleagues for a few years and afterwards he could soon pick up the threads of his profession; do the same to a scientist and his whole career may be ruined and others' impoverished.

The human rights legislation that has accumulated since the 1948 United Nations Universal Declaration of Human Rights is extensive, and the report provides a valuable guide. Of central interest to scientists are assertions such as that of the United Nations Declaration: 'Everyone has the right to freedom of opinion and expression; this right includes freedom to hold opinions without interference, and to seek, receive and impart information and ideas through any media and regardless of frontiers.' The problems, however, arise in the qualifications. The United Nations Declaration is only proclaimed as a 'common standard of achievement'—highly desirable but not legally binding. A document which binds its members and which provides a mechanism for redress—the European Convention on Human Rights—says almost the same thing, but it then goes on to add 'the exercise of these freedoms . . . may be subject to [legal and democratic] restrictions . . . in the interests of national security, territorial integrity or public safety, for the prevention of disorder or crime, for the protection of the reputation or rights of others, for preventing the disclosure of information received in confidence, or for maintaining the authority and impartiality of the judiciary'.

Some of these restrictions, the report points out, do no more than prevent anyone who does things like shout 'Fire!' in a crowded theatre from avoiding the legal consequences by claiming freedom of expression. But there are plenty of murky areas connected with 'national security', 'protection of morals' and so on, and it is a disappointment that the study group, having noted these areas, did not pause at such a point and devote rather more than three paragraphs to a discussion of the subject. Also, having drawn attention to differences in priorities between countries operating different economic systems, the group could well have elaborated rather more on the problems of misunderstanding between countries which rate civil rights high but give less guarantee of economic benefits and those which have an opposite order of priorities. There is one paragraph in the report in which it suddenly seems possible that this question will be analysed in depth, but the opportunity is passed over.

But what should scientists do when they hear of violations of human rights? We have not just an internationally accepted right to protest, says the report, but a duty to do so, and in a public way both individually and through organisations such as learned societies. Private persuasion is allowed its place but the major sanction 'must always be that of public protest'. The clear intention of the report thus emerges in its last few pages—bodies such as the Royal Society have no business steering clear of public pronouncements on the oppression of scientists.

Not for the first time, however, the Council for Science and Society has almost predetermined the conclusions of a study group by its choice of membership of that group. We greatly admire the vigour of John Ziman, convener of the group, and the integrity of its members—though it hardly seemed necessary for them to write that they all 'share a high degree of commitment to the ideal of the relentless and objective search for knowledge, wherever it may lead'. But was the outcome ever in doubt? Professor Ziman's views are well known on the subject of Royal Society intervention, and this makes the report's conclusions seem less weighty than they would have been had the Council gone to someone less committed to convene the group. The excellence of the sections on human rights law and on the needs of scientists remains undiminished. They should be widely read, and the hope must be that the document will have some effect. But its conclusions, put together a little skimpily as if they were self-evident, lack the coresponding weight. □

Peace research: the whys and the wherefores

Frank Barnaby, head of the Stockholm International Peace Research Institute (SIPRI), outlines the course being followed by military technology and offers his view on the role of peace research

"WITH the continuing technical progress and the current world-political constellation, it is highly probable that there will be an atomic world war before the end of this century." This statement was recently made by Professor Carl Friedrich von Weizsäcker, the West German physicist-philosopher, Director of the Max-Planck Institute in Munich, and author of the widely-discussed book *The Relevance of Science*. According to Professor von Weizsäcker a nuclear world war will probably be followed by the establishment of a world government which will ensure that no more nuclear world wars occur. In his view, then, it is likely to be the Third World War which will end all world wars.

Most students of military technology would agree that the probability of a general nuclear war is increasing—at least if only the consequences of advances in and the spread of military technology are taken into account. Three specific factors contribute to this conclusion. First, the nuclear arms race between the USA and the USSR could (and, therefore, probably will) eventually lead to a first-strike capability. This does not necessarily mean that one side will be able to destroy completely the other's capability to inflict casualties and damage in retaliation for a first-strike, but rather that this capability may be perceived to be sufficiently reduced to limit the casualties and damage to a level that might be considered acceptable for a given political goal. This level will depend on the recklessness and adventurousness of the political and military leaders in power at the time.

First-strike strategic nuclear weapons are likely to result from the improvement in accuracy of ballistic missiles. The accuracy of the current US Minuteman III land-based intercontinental ballistic missile, for example, is about 400 metres, at a range of about 13,000 kilometres. By the mid-1980s warheads with accuracies of about 30 metres may be available which, by any standards, will be first-strike weapons, even against small hardened targets. When it is remembered that the German V-2 missile could barely be depended on to hit London, the rate of progress which has been and which is still being made in missile technology will be realised. And similar progress has, of course, been made in many other areas of military technology.

If the limit has nearly been reached in the performance of strategic offensive missiles, there is still some way to go in strategic defensive systems. Much research is under way in defensive weapons against ballistic missiles and in anti-submarine warfare. A breakthrough in these areas, which can by no means be discounted, would be extremely dangerous for world security.

When the political and military leaders perceive a first-strike capability to be near they may intensify measures to defend the population, and to protect industry and vital plants from nuclear explosions. The effectiveness of these measures will of course be taken into account when assessing first-strike capabilities. That perceptions about "winning" a strategic nuclear war are likely to be proved wrong, if ever put to the test, is hardly a consolation, since civilisation as we know it will be destroyed in the process.

The second reason for concern is the worldwide spread of the capability to produce nuclear weapons, through the spread of peaceful nuclear technology, and of the technical knowledge and expertise required for nuclear-weapon production. It is extremely unlikely that any new international measures to establish a viable non-proliferation regime will succeed where the Non-Proliferation Treaty has failed. The most that can be hoped for is that measures—such as moratoria on the reprocessing of reactor fuel elements to remove the plutonium from them and on the large-scale construction of breeder reactors—will be taken to slow down the rate of proliferation.

The Achilles heel of the proliferation problem is that sufficient fissile material for a modest nuclear force could be obtained, by virtually any country wishing to do so, on a small scale, and secretly. The components for a small reactor able to produce enough plutonium for say two atomic bombs per year are available on the open market. And the reprocessing of a few tens of kilograms of plutonium annually could be done on a laboratory scale. The indigenous clandestine construction of a small uranium enrichment plant is also within the capabilities of many countries.

It is already hard to know for certain whether or not some countries have, or are producing, nuclear weapons (Israel, for example, has kept the world guessing for years), and this uncertainty will increase with time.

The third factor is the international trade in arms which is spreading the most sophisticated conventional arms worldwide. Since the October 1973 Middle East war this trade has increased at an unprecedented rate and is now virtually out of control. As a consequence, the arsenals of some Third World countries are more up-to-date than are those of some industrialised countries. Many of the aircraft and missiles supplied to the Third World are capable of delivering nuclear weapons (on today's standards, even a crude nuclear weapon with an explosive power equivalent to that of 20,000 tons of TNT should weigh less than 1,000 kg).

Some of the major recipients of arms become strongly bound to the supplier state, often to an extent hardly distinguishable from that of a close ally. This dependence is considerably heightened by the knowledge that in modern war munitions — particularly anti-tank and anti-aircraft missiles—are normally used at a very high rate. Victory may depend on the willingness of a great power to provide the massive quantities of replacements needed throughout the war. In this sense, the great power often becomes, implicitly or explicitly, the guarantor of the client state.

A war in, for example, an unstable region, involving one or more of these client states, could escalate from a conventional war to a limited nuclear war fought with the nuclear weapons of the local powers and then to the involvement of the great powers, committed to defend their clients—an involvement which might end in a general nuclear war. This end result is, of course, most likely to occur if one of the great powers perceives the chance of making a successful first-strike.

While not disputing the dangers inherent in unrestrained military technology, there are those who argue that increasing cooperation between states —in, for example, economic affairs—will prevent the great powers from pursuing policies which lead to a general nuclear war. Whether or not tendencies for cooperation will, in the next two or three decades, outweigh those for confrontation is a matter of personal judgment. I find it hard to be optimistic given the rapid rate at which military technology advances and the relatively slow rate at which effective bonds between nation states are formed.

Taken together, the three factors

will produce an extremely dangerous period in the not too distant future, unless steps are taken to prevent it. If, for example, a first-strike capability is developed it will take a very strong-willed and responsible leadership to resist arguments for its use. With so much at stake for us all, can we really afford to sit back and hope that the right men will be in power at the crucial times?

There is no reason why the current rate of progress in military research and development, the activity mainly responsible for progress in military technology, should not be sustained. In fact, because R&D is the most difficult of all military activities to control, one can expect the rate of progress to continue to increase. Military technology has indeed come a very long way since the Second World War. Where will this technology take us by the end of this century? Scientists who ponder this question are generally doomsayers.

All of the ramifications of advances in military technology are known only to an extremely small group of people outside governments—to a few hundred scientists worldwide. It is a sobering thought that even within the world's governments at most a small handful of political leaders and their advisers are aware of the consequences of the onward rush of military technology. Under these circumstances and given the catastrophic nature of nuclear war, are the few scientists who do know the facts under a special obligation to publicise them?

The social responsibility of scientists has been much discussed over the past twenty years but inconclusively. There does, though, seem to be wide agreement that scientists do at least have a duty to inform non-specialists about any scientific developments (or the application of research results) which could lead to adverse social consequences. And the more dire the consequences then presumably the greater the duty to inform. But to whom should the information be addressed? In what form should it be presented? And how can its dissemination be best achieved?

These questions are particularly relevant to the Stockholm International Peace Research Institute (SIPRI), an independent institute established in 1966 and financed by the Swedish Parliament, which publishes factual studies on military technology, armaments, and disarmament (see *Nature*, **246**, 397–400; 1973). During SIPRI's lifetime the situation in armaments and disarmament has changed considerably. Ten years ago there was still reason to hope that arms control negotiations would succeed in stopping the Soviet-American arms race and lead, step-by-step, to significant disarmament. We now know differently. Throughout this period, the arms race has continued virtually unconstrained and the only weapons destroyed by international agreement have been biological weapons — weapons of little military interest.

Arms control may lead to the management of the arms race. Arms control may contribute to Soviet–American détente. But, other than the actual negotiators and committed political leaders who soon develop a high level of 'professional optimism', few now believe that the arms control approach will actually lead to disarmament.

> 'There is no reason why the current rate of progress in military research and development, the activity mainly responsible for progress in military technology, should not be sustained. In fact, because R & D is the most difficult of all military activities to control, one can expect the rate of progress to continue to increase'

In my opinion, this lack of success, and the resultant ever-increasing probability of nuclear war, demands urgent action. Whereas in the mid-1960s it seemed that SIPRI's research should be aimed at the specialists advising those decision-makers and diplomats able to affect arms control negotiations, it is now clear that a much wider audience must be reached.

The political leaders of the great powers clearly lack the will to negotiate nuclear disarmament. There are two possible ways of applying the necessary pressure on these reluctant politicians. One is through public opinion, or at least the most influential sections of it, and the other is concerted action by the smaller countries.

SIPRI has neither the skills nor the resources needed to appeal directly to the public. But it can, and does, provide information in a form easily used by those who have — particularly journalists working for the press, radio and TV. SIPRI also makes sure that its material reaches the relevant authorities in the smaller powers.

A few of these powers—Canada, Mexico, the Netherlands, Sweden and Yugoslavia, among others—have a sizeable group of officials well trained in disarmament and related matters. But the bulk of the smaller countries do not. The one or two individuals in these countries dealing with the subject need information in a summarised and easily digested form which should also give a sense of the urgency of the current situation. An authoritative institute is able, and most would say has the duty, to provide this crucial service in addition to its various research publications.

All of this pre-supposes that far-reaching disarmament (beginning with nuclear disarmament) can be directly negotiated in today's world of 150 or so states using the traditional methods of diplomacy and international law. SIPRI believes this to be so. SIPRI also believes that unless this process is soon begun it may be too late to prevent a nuclear world war.

Some peace researchers, however, reject these beliefs. They argue instead that conflict—leading eventually to general nuclear war—is inevitable because of the very nature of the present international system. The only way to prevent this, they say, is to change totally the system by developing entirely new international political and social institutions. In their view it is unrealistic to expect today's world to disarm, and even if it did the likelihood of nuclear war would remain. In my opinion, this approach is not only incorrect but dangerous. There is probably insufficient time to produce a new world order, even if such a thing were feasible. And there is the danger that too much emphasis on the inevitability of nuclear war may make it a self-fulfilling prophecy.

In summary, I believe that the dissemination of objective information on armaments and disarmament to specialists is still crucial but it is not enough. In addition, it is necessary to explain to non-specialists where military technology is taking us—particularly to the groups mentioned above—in a way which will stimulate their interest and hopefully provoke them to action. In this sense, SIPRI's work has become somewhat more 'political'.

The fear that the presentation of material in a brief and readable (even somewhat enlivened) form must damage the reputation for objectivity of a research institute is, in my opinion, unfounded. When the need for political effectivity is urgent and great it is even defeatist. ☐

Czechoslovakia (1)

The general . . .

A Special Correspondent discusses the climate in which science operates in Czechoslovakia

THE fact that by comparison with Czechoslovakia probably no other state in the world has so many members of its political leadership with the highest academic qualifications seems to express a very attractive idea—namely, that educational opportunity is equal for everyone, that political leadership emerges in a democratic way and that people make rational choices for the best of the society. The logical conclusion that those who take decisions are the most competent is undermined by two factors.

First, in practice it is the Communist Party which is the repository of power. For example, only those scientists who conform to party rulings are acknowledged as scientists. And the acquisition of school qualifications or of a university degree and high academic honours in the space of months may well accompany a person's improving political fortunes. Still, the fact that political leaders regard it as worthwhile to present themselves as academics symbolises the significance which they attribute to the community of learning.

Secondly, some prominent intellectuals in Czechoslovakia are now in revolt against the present regime. The *Charter 77* human rights manifesto, originally signed by more than 200 persons of "various shades of opinions, faiths, and professions", included some 25 scientists and doctors. It has since been countersigned by at least another 200 supporters, and contains a number of accusations of relevance to all who value academic and scientific freedom (see box).

The current Five Year Plan (1976–80) shows clearly that the present leadership continues to hope for an important contribution of science to economic development. Expenditure for research and development is to grow by a third of the present sum to 3.8% of GNP in 1980, and the proportion of people working in science and technology is to be 21 out of every thousand working in general. Expenditure on equipment has nearly doubled in the past five years and is to grow still further; so will the salaries of scientists.

The significance attributed to science by the present regime in Czechoslovakia is not just theoretical. Yet the apparently grave difficulties achieving the expected results from the effort put into science seem more and more clearly to arise from the problems inherent to the system itself and not just from the capacities of its leadership.

Of the two different views about the preconditions for the advancement of learning—one stressing free inquiry, the importance of competition and the natural selection of ideas, the other maintaining that an attainment of shared norms and procedures by the scientific community is desirable and that scientific effort as a whole can and should be rationalised—communist ideology is decisively in favour of the second view, since it believes that the development of science can be encouraged when planned. Virtually the whole of scientific effort is subject to state planning in Czechoslovakia.

Even though science is administratively subordinate to the central agencies of government, it would be misleading to say that one identifiable centre prescribes what should be done. Short-term plans spell out work assignments for practically every scientist; this is done on the basis of two-yearly surveys of the country's material and manpower resources in science. The latest of the regular five-year plans was worked out by 800 scientists and specialists from production, directors and representatives of the relevant working places and, of course, government ministries. Political supervision comes only through the political bounds on the participants themselves. Occasionally mention is made of preparations for a long-term plan, but so far nothing has materialised.

One of the basic difficulties is the right balance betwen research and development assignments. The current five year plan for Czechoslovak science foresees 346 main tasks; 70% of these are oriented towards current technical developments, 23% are concerned with other social needs, such as medicine and social science, and 7% are tasks of pure research. The proportion of uncommitted to committed goals is, even in the country itself, considered low.

One problem frequently pointed out in Czechoslovakia is the highly unnatural age-structure of people working in research. The official figure for the average age is 45, but this does not express the fact that there is a whole generation missing. After the continual purges in the early 1970s and the emigration of many highly qualified scientists to the West, the number of people between 30 and 50 diminished drastically. There are institutes where only isolated persons survived the past few years. The zigzag course of Prague's present policy has meant alternating periods of relative reconciliation and dogmatic reassertion. Changes and deviations from the science plan require a very expansive, unproductive bureaucracy, and the administrative complex is very demanding of scientists' time—especially when taken together with permanent political courses and meetings.

'A virtual apartheid'

Some extracts from the *Charter 77* human rights manifesto:

•

The right to freedom of expression . . . is in our case purely illusory. Tens of thousands of our citizens are prevented from working in their own fields for the sole reason that they hold views differing from official ones, and are discriminated against and harassed in all kinds of ways by the authorities and public organisations. Deprived as they are of any means to defend themselves, they become victims of a virtual apartheid.

. . . . countless young people are prevented from studying because of their own views or even their parents'. Innumerable citizens live in fear of their children's right to education being withdrawn, if they should even speak up in accordance with their convictions.

Any exercise of the right to 'seek, receive and impart information and ideas of all kinds, regardless of frontiers, either orally, in writing, or in print' . . . is followed by extra-judicial and even judicial sanctions . . . •

No philosophical, political, or scientific view or artistic activity that departs ever so slightly from the narrow bounds of official ideology or aesthetics is allowed to be published.

Many scholars, writers, artists and others are penalised for having legally published, years ago, opinions which are condemned by those who hold political power today.

All national institutions and organisations are in effect subject to political directives from the machinery of the ruling party.

Clause 2, article 12 of the [International] covenant [on Civil and Political Rights] guaranteeing every citizen the right to leave the country is consistently violated or under the pretence of 'defence of national security' is subjected to various unjustifiable conditions . . . The granting of entry visas to foreigners is also treated arbitrarily, and many are unable to visit Czechloslovakia merely because of professional or personal contacts with those of our citizens who are subject to discrimination.

Czechoslavakia (2)

. . . and the particular

Vera Rich considers the position of scientists in the Slovak Republic

SOME Western analyses of the Soviet and East European interest in detente stress their need for modern technology. Insufficient modernisation of industrial production in these countries is said to be due to the difficulties in implementing new technologies. The problem may not be a simple mechanical one, though; it may also be related to the relegation of science to the status of a mere tool whose direction has been predetermined by the needs of society.

One of the main tenets of science policy throughout the Comecon block is the tightening of links between research and industry. The place of science in the economy is assessed primarily for its practical and immediate benefits. In the case of a relatively newly-developed area such as Slovakia, which for many years was the 'backward' partner of the Czechoslovak federal state, this can mean that scientists bear responsibility for deficits due largely to planning errors and past neglect.

Slovak research scientists have recently been blamed for deficiencies in the local metallurgical and engineering industries. This involves some 40% of all research workers in Slovakia, or more than 15,000 persons. According to the November 1976 meeting of the Central Committee of the Communist Party of Slovakia, "the slow introduction of new ideas in production and the contribution of research institutes so far are not satisfactory." There is a high turnover of leading technical and managerial personnel, weaknesses in planning and leadership, and a general failure to use modern equipment to the best of its ability.

Federal planning aims to "balance the sociopolitical and economic standards of the Czech and Slovak republics" and the development of Slovak industry is a major theoretical issue. The need for such a development is reflected in the targets for the present Five Year Plan: overall Czech industrial production is to be increased by 32.3% and Slovak production by over 44%. This means that output from Czech industrial enterprises must rise by 48.5% and from Slovak enterprises by 70%. The targets are a 50% rise in Slovak exports with engineering exports in particular rising by 100%. It is difficult to imagine that all these figures will be met, particularly as Slovakia's main 'export' products—steel and aluminium—are not in fact exported in ingot form, but go to Bohemia to be manufactured into finished articles, which are then credited to the Czech Republic.

The slow growth of Slovak industry is partly historical in origin—when the Czechoslovak Republic was set up by the Treaty of Versailles, the Czech lands, which had been under Austrian rule, already had flourishing industries, while Slovakia, which had formed part of the kingdom of Hungary, was largely agricultural. The policy of the First Republic was to maintain this *status quo* thereby causing considerable Slovak resentment against the federal government. During the late 1940s the industrialisation of Slovakia was a major issue. Students uncertain of which subject to pursue were urged into technology, in particular metallurgy.

One of the first actions of the new communist government was to start planning the vast East Slovak Iron Works at Košice. This was primarily a political decision: the only resources the area could offer were limestone for fluxing and a large labour force. Iron ore had to be brought from the Ukraine and coke from Czech Silesia. One of the charges bought against Slansky, the former Party Secretary, during his trial in 1952 was that this was an "impossible" scheme, and construction at the plant was halted for three years. In 1968, when open discussion became possible under the Dubček regime, it was revealed that steel produced at Košice was costing 8–10 times that produced in Japan, although Japan was shipping iron ore from Australia and Brazil and coke from Australia. Since 1968, the Husak government has invested sums in Slovak industry which are out of all proportion in comparison with the population of the republic, causing some resentment from the Czechs. The Slovaks, however, stress the need for even more investment. In September, 1975, Miloslav Hruskovic, the Secretary of the Central Committee of the Slovak Communist Party, noted that "serious problems persist in the capital investment plan", and that scientific and clinical development has not been "reflected in investment construction as much as it should have been" (no details were given).

Camera Press

Gustav Husak, Communist party head

Improvements

Certainly investment has produced considerable improvements. During the 1971–75 period industrial production in West Slovakia (mainly chemical and engineering) is said to have risen by 54.6%, while in East Slovakia the "volume" of production increased by 66.2%. There was considerable investment in the chemical works at Bratislava, and the notorious Košice ironworks now has the second highest output of all Czechoslovak metallurgical plants, and the lowest manufacturing costs (no details given). And although the Federal reports indicate a general lack of enthusiasm and expertise in the use of computers, particularly for information and planning work, the development of a scientific and technical information system in Slovakia was, according to the Slovak Minister of Development and Technology, J. J. Busa, going forward well. The new arrangement, by which Cuba will supply the Comecon countries with nickel will undoubtedly benefit the nickel-works at Sered, which until now has had to use ore from Albania.

Nevertheless, according to Hruskovič, what is needed is a greater intensification of production, together with a more economic use of raw materials, commodities and energy and an improvement in quality and technical standards. The Central Committee of the Slovak Communist Party proposes a stick-and-carrot policy for scientists and technologists: those who "have the courage to take risks on the challenging part of technological progress" will receive "moral rewards" and sharply-differentiated "material incentives" unhampered by "unwarranted egalitarianism, because this slows down progress". Those, however, who cannot "cope with the tasks of the present", should be transferred to other posts "tactfully, and before it is too late". ☐

BRAZIL

A growing resistance

Bruce Handler reports from Rio de Janeiro on recent developments involving the controversial German–Brazilian nuclear deal

Attempts by the Carter Administration in the United States to undermine the controversial nuclear technology agreement between West Germany and Brazil are resulting in precisely the opposite of what Washington wants. Brazil are resulting in precisely the go through with the atomic pact. Formerly divergent internal currents in Brazil are rallying behind the Brazilian government in defence of the agreement with Germany And other Latin American countries—especially Argentina, which has always been considered Brazil's principal rival—are sticking up for Brazil in an unusual display of Latin American solidarity.

Part of Jimmy Carter's election campaign platform consisted of promises to oppose the spread of nuclear technology, especially in the underdeveloped Third World. And just a few days after taking office, he dispatched his Vice President, Walter Mondale, to West Germany to ask Chancellor Helmut Schmidt to reconsider German plans, under the nuclear agreement, to aid Brazil in developing its own full-fuel cycle. This German knowhow is to allow Brazil to enrich its own uranium for use in electricity-producing nuclear reactors, but it also will make Brazil theoretically capable of producing plutonium—a key ingredient in nuclear weapons

After some initial backtracking, however, Germany said it will go ahead with the Brazilian agreement as planned. Brazil declared flatly that it "sees no possibility" of altering or delaying the pact, as the United States wants. And Paulo Nogueira Batista, the head of the Brazilian government's nuclear power company, Nuclebras, told the press: "The agreement is being carried out on schedule, and in some cases you could say we are making even more progress than we expected."

Brazil's foreign minister, Antonio F. Azeredo da Silveira, did say later, though, that Brazil is willing to discuss "global aspects" of the problem of the spread of nuclear technology This, the minister noted, ought to prove to President Carter that Brazil intends to act like a responsible, adult nation Azeredo da Silveira invited the US Secretary of State, Cyrus Vance, to Brazil for talks, and it appears Vance will come.

The attempted pressure-play from Washington resulted in a remarkable outpouring of unified internal support for Brazil's authoritarian, military-run government, which had pressed hard to get the nuclear agreement with Germany in the first place The actual agreement was signed in mid-1975, but the Brazilians had begun secretive negotiations with the Germans several years earlier.

Marcos Freire, a senator from the sole opposition political party permitted in Brazil, said "The United States has no moral authority to interfere with the nuclear treaty." He speculated that "powerful economic interests" might be behind Washington's meddling, in view of the fact that the total value of contracts to be signed under the Brazilian–German agreement is conservatively estimated at $5,000 million

Another member of the opposition party, Representative Joao Cunha, said Brazilian President Ernesto Geisel, "speaking in the name of all Brazil's 110 million citizens, should repudiate this US manoeuvre."

Even Brazil's academic and scientific community, which under the current government tends to stay as far away from politics as possible, has spoken up with regard to the nuclear agreement. "This government is correct in not permitting outside interference in affairs over which it has exclusive sovereignty," said Professor Marcelo Dami, a physicist from Sao Paulo. Dami made it clear that he did not like the secretive way in which the government had gone about negotiating the agreement with the Germans and also that he has reservations about the type of fuel that was chosen for atomic reactors that are to be built in Brazil under the pact. "But disagreements of this nature now must be put in the background, in view of higher national interests that are at stake," he said.

Helio Jaguaribe, a political scientist whom some members of Brazil's military regime regard as a "dangerous intellectual," also came out in full support of the government. "President Geisel deserves the warmest applause from all Brazilians for the firm position he has taken in supporting the agreement," he declared "President Carter's concern about the proliferation of nuclear weapons is fully valid But Brazil has no nuclear weapons, and it does not intend to have them."

He said a distinction has to be made between stopping the proliferation of nuclear weapons and "the desire of the superpowers to maintain a monopoly on nuclear technology" The Brazilian government, furthermore, was "absolutely right in not signing the Nuclear Non-Proliferation Treaty," since to do so "would perpetuate the underdevelopment of third world countries."

Unexpected defence for Brazil came from across the border in Argentina, which until now has been wary, to say the least, of its fast-developing neighbour's nuclear energy programmes and goals Argentina began its own nuclear programme in the 1960s and was regarded as the most important country in Latin America in terms of atomic development until the German–Brazilian deal emerged.

"Argentina supports the Brazilian government in the nuclear field," declared the newspaper *La Opinion* of Buenos Aires, which is said to reflect the official line of President Jorge Videla's military-run regime. "The US offensive against Brazil, which is aimed at stopping progress on its plan with Germany to install eight more nuclear reactors, a uranium enrichment plant and a fuel reprocessing plant, is a blow which could be fatal to Brazil's nuclear industry What happens to Brazil today could happen to Argentina tomorrow."

And in a statement that must have surprised a lot of Brazilians, *La Opinion* wrote: "Brazil is not just any developing country It is a co-participant in the destinies of Argentina, in the Latin American context."

Amid the renewed debate surrounding the Brazilian–German nuclear pact, Brazilian government officials have hastened to repeat this country's justification for seeking the agreement in the first place.

- Brazil produces only 20% of the crude oil it needs every year. Its domestic coal is of low quality. Its remaining major potential hydroelectric sources are in the Amazon jungle, thousands of miles away from its centres of population and industry Therefore, Brazil must develop nuclear energy as a source of electricity
- Brazil must possess the knowhow for supplying itself with nuclear fuel. Despite a US offer to furnish already processed enriched uranium (with no transfer of technology) for Brazilian reactors, Brazil cannot run the risk of remaining dependent on foreign sources for energy (Look what happened to the United States during the Arab oil boycott, a much heard Brazilian argument goes.)
- Brazil is a peaceful country It doesn't need or want an atom bomb, and it would not divert resources from vital social and economic needs to build one. And besides, the safeguards in

the German-Brazilian nuclear agreement were approved by the International Atomic Energy Agency, with both the United States and the Soviet Union voting in favour.

Although none of the reactors or fuel processing equipment specified in the nuclear agreement actually has been built yet, the accord most definitely is being carried out, as the head of Nuclebras, Nogueira Batista, said.

More than 100 Brazilian scientists are taking specialised training in Germany, and the Brazilian government has allocated generous amounts of funds to local universities to expand programmes in nuclear-related fields of science. German atomic technicians already are working in Brazil. Adverts in newspapers are seeking secretaries fluent in Portuguese and German. Six subsidiary firms have been set up under Nuclebras to implement the agreement. These companies are to operate in areas such as nuclear technology and engineering, heavy equipment manufacturing, uranium prospecting and mining, and isotope enrichment.

Financing arrangements already have been completed for the first two 1.3 million-kilowatt German reactors that will be built in Brazil, with knowhow and components to be supplied by Germany's Kraftwerke Union. Related contracts have been signed between other German and Brazilian companies and consortiums, private as well as governmental, for site clearing and preliminary building construction.

Unofficial calculations show that over $1,000 million-worth of cruzeiros, marks and dollars have been spent or committed under the German–Brazilian nuclear agreement so far. ☐

USA

Talk about the weather

Unusual weather encourages discussion of weather modification. Colin Norman reports on the debate in the western United States

THE strange weather patterns which have brought freezing temperatures and record snowfalls to the eastern United States this winter have brought the opposite problem to many western states. A severe drought is threatening agricultural production in California and it may disrupt hydroelectric power supplies in the Pacific northwest. And in the Rocky Mountain states the snow cover is so thin that the spring runoff is unlikely to be sufficient for crop irrigation, ranching, industry and domestic needs. In short, while the east is bracing for possible floods when the thaw comes, the west is facing a debilitating drought.

One consequence of this topsy-turvy pattern is that considerable public interest has been rekindled in the use of weather modification techniques to try to increase snowfall on the western mountains before spring arrives. The most dramatic expression of such interest came in Colorado earlier this month, when the state legislature approved a bill to provide up to $250,000 for cloud seeding operations in the state. A major effort is also under way in Utah, some seedings have taken place in California, Oregon and Washington state, and an extensive long-term weather modification programme is also under discussion for the upper Colorado basin. One product so far is the generation of a good deal of local controversy.

This year's anomalous weather patterns have been caused by a stationary ridge of high pressure off the west coast, which has disrupted the normal westerly winds. Usually, the prevailing westerlies bring winter storms in from the Pacific to dump snow on the mountains in the western states, but this year the winds have been deflected northward and have looped through Canada to descend on the eastern United States. The consequences in the east have ben dramatic; in the west they have been more subtle.

Very little rain or snow has fallen in the Pacific northwest since September, and some industries which depend on hydroelectricity may face power reductions of 25% by the end of this month. Fire hazards abound in forest areas which are usually under several feet of snow at this time of the year. In California, domestic water supplies are already being rationed in Marin County near San Fransisco, and last week the Bureau of Reclamation (an agency in the Department of Interior) notified farmers in the San Joaquin Valley that their irrigation water may be cut by 75% later this year. California produces about 40% of the fresh fruits and vegetables consumed in the United States. In Utah and Colorado, snowfall has been about 30–40% of normal, and as a result ranchers are selling off cattle because they cannot get sufficient feed and the winter wheat crop may be in jeopardy.

Another, more distant problem is likely to arise because spring runoff from the Colorado Rockies supplies most of the water in the Colorado River, which in turn provides much of the irrigation and drinking water for parts of Arizona, California, Nevada and northwestern Mexico. Though water now in storage in Lake Powell behind the Glen Canyon Dam and in Lake Mead behind the Hoover Dam will be sufficient to meet downstream needs for at least a couple of years, if this year's weather patterns are repeated next winter, some serious problems could occur. Small wonder, therefore, that weather modification techniques are attracting attention. They will not provide salvation, however.

The legislation in Colorado will provide funds for massive cloud seeding operations during the rest of this winter on the western slopes of the Rocky Mountains, using silver iodide generators on the ground. Some airborne seeding will also take place in the eastern plains. In Utah, routine cloud seeding, sponsored by the Utah Water Conservation Board, has been undertaken for the past three years, and this year it is being expanded to cover nearly two-thirds of the state.

It should be noted, however, that cloud seeding can only increase natural precipitation; it cannot create snow when the conditions are not right for snowfall. Thus, the Colorado and Utah cloud seeding efforts will only be conducted if westerly winds return to bring snow clouds to the Rockies. According to a spokesman for the Colorado Water Conservation Board, the seeding programme will, at best, increase spring runoff this year by about 200,000 acre feet, an amount which will certainly not wipe out the anticipated shortages.

In a normal year, spring runoff from the Colorado Rockies into the Colorado River amounts to about 11 or 12 million acre feet. This year, Colorado officials are estimating that the runoff will be down to about 7 million acre feet.

Although any contribution from cloud seeding would be a help, environmentalists in Colorado vigorously opposed the emergency legislation when it was under consideration in the state legislature. Kevin Markey, a representative of Friends of the Earth, said last week, for example, that "it isn't emergency cloud seeding legislation, it's emergency political legislation to let everyone know that the governor and legislature are doing something". Markey argues that the time and effort spent on cloud seeding would be better devoted to water conservation programmes.

Environmentalists are particularly concerned about plans for a possible massive cloud seeding effort now under discussion in Colorado and Utah, which would entail regular seeding of winter storm clouds to try to increase spring runoff to the Colorado River each year and boost water supplies to the arid southwest. A pilot-scale seeding effort was conducted by the Bureau of Reclamation in southwestern Colorado between 1970 and 1975 to measure the impact of cloud seeding on snowfall, and to evaluate potential long-term environmental consequences of a major effort covering most of the state. The results of that pilot effort were released last month, and they are adding to the debate.

The pilot project came up with two seemingly contradictory conclusions. First, it indicated that regular seeding may increase the snowpack by about 10%. And second, it seemed to show that there was no statistical difference in the amount of snowfall on days when seeding took place compared with days of natural snowfall. Officials in the Bureau of Reclamation explain the contradiction by arguing that the seeding sometimes increased snowfall outside the target area, and residual silver iodide in the air often increased snowfall on days after seeding took place. Some seeding also took place on days when the conditions were not right for snowfall, which masked the overall effect, they claim. The ambiguous results are adding fuel to the debate about the advisability of sinking large sums into seeding this year, however.

The pilot project ended in May 1975, and discussions are now under way between officials in Utah and Colorado and representatives of the Bureau of Reclamation over the possibility of expanding the effort into a large-scale demonstration programme. The Bureau has no money in its budget to begin such a project, however, and the earliest it could be started would be the winter of 1978–79. In the meantime environmentalists, who fear possible long-term effects on wildlife, increased damage from avalanches and potential effects on rainfall downwind from the target area, will continue to urge that water conservation be supported instead.

Whatever the outcome of that debate, it will not help alleviate the anticipated drought this spring. That would require at least twice the average snowfall between now and April, a prospect which weather forecasters deem extremely unlikely. As Colorado's Governor, Richard Lam, stated when he signed the emergency cloud seeding legislation, "It's a roll of the dice". ☐

BRITAIN

Solar commitment

Britain moved another step in its efforts to implement a broad national energy policy with the announcement last week of a programme supporting the exploitation of solar power. Chris Sherwell reports

A POSITIVE but measured commitment to solar energy from the UK government; a positive but measured response from the country's solar energy lobby. That was the state of play at the end of last week after the Department of Energy (DEN) had unveiled its latest research and development programme, this time in the field of solar energy.

The announcement, neatly preceded a day earlier by a DEN junior minister's well-publicised visit to a solar heated house to reaffirm the commitment to explore alternative energies, was timed to coincide with the long-delayed publication of a report prepared for the DEN by its Energy Technology Support Unit (ETSU), based at Harwell[*]. In following earlier announcements in support of wave and geothermal power it marks another step towards comprehensive exploitation of renewable sources of energy. Reports on combined heat and power and on tidal energy are expected in the next couple of months.

The new commitment of resources to solar energy amounts to £3.6 million over four years, and is expected to bring total government expenditure in the area to about £6 million over the same period. The £3.6 million will be concentrated on three principal activities: the use of solar energy for water and space heating (the ETSU report sees this as offering the best return); the collection of additional data on solar energy, and the biological conversion of solar energy.

DEN officials were reluctant to indicate precisely how it would be allocated. An important fraction will go to work on biological conversion, but the bulk of the money will be used to accelerate the development of suitable solar power appliances. The DEN's idea is to negotiate contracts with companies with satisfactory proposals, offering them up to 50% funding to reduce the risk involved and encourage the work.

Much of the support will also complement work done by the Department of the Environment, notably through the Building Research Establishment. DEN funds will not be used to finance R&D on photovoltaic devices, however. This is already being done under the aegis of the Department of Industry.

The International Solar Energy Society in Britain (UK–ISES), though welcoming the latest development as a "good starting point" and though in agreement with the balance struck by the DEN, points out that the UK commitment compares unfavourably both in amount and in time-scale with that in other countries.

• The UK–ISES also points out that in its report released last year, *Solar Energy a UK assessment*, it estimated that solar energy could make an energy contribution of 35 million tonnes coal equivalent (mtce) by the year 2020—about 10% of total energy needs. It urged an immediate incremental budget devoted to solar energy, beginning at £2 million a year in 1976 and reaching £10 million by 1980.

Dr Walter Marshall, the DEN's Chief Scientific Adviser, citing the ETSU report, said solar energy could be making a contribution of "around 2% of our present requirements" within 25 years (some 7 mtce), an amount which could represent a "take-off point" and might be increased by a factor of about 10 over the ensuing 25 years or so. But it was hard to assess the real potential, he said, since the trends in fuel prices weren't known.

Dr Marshall paid tribute to the UK–ISES for the quality of its report. And in response to a question about the delay in the government's announcement of a programme and in publication of the ETSU report—research for it was completed 20 months ago, in July 1975—he acknowledged that he had made a mistake in not setting the wheels turning in the DEN in anticipation of a favourable verdict.

That seemed like a surprising admission. But in a country blessed with a surfeit of energy for the medium term from its oil, gas and coal resources and its existing nuclear programme, the error of judgment seems more understandable. Still, the UK–ISES emphasises the opportunities even to an energy exporter offered by solar energy investment—especially in the field of export.

The UK–ISES's enthusiasm for solar energy is not based just on faith. In January alone it received five telephone calls a day and 253 written enquiries from non-members. The newly-won enthusiasm of the DEN cannot be so complete. There the real fight is between coal and nuclear power. ☐

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[*] *Solar energy its potential contribution within the United Kingdom* (London, HMSO, £3.00)

IN BRIEF

EEC safeguards progress

The complicated checking agreement between Euratom and the International Atomic Energy Agency (IAEA), reached some time ago, is now in force. With the exception of France, EEC members are now coordinating the application of IAEA and Euratom safeguards to their nuclear plants, and routine IAEA inspections will take place at the same time as Euratom inspections. There is a possibility that France will enter into its own trilateral agreement with the IAEA and Euratom. The progress on safeguards, announced last week, is expected to reduce the threat to the EEC's supplies of enriched uranium from North America. Officials in Toronto were reported last week as saying that Canada may soon resume uranium shipments to Europe.

Spacelab choice made

The European Space Agency (ESA) and NASA have chosen the 77 scientific and technological experiments to be carried on the first Spacelab mission which is scheduled for the second half of 1980. Available resources will be shared equally by NASA and ESA—61 of the experiments are European, 15 are American and one is Japanese. The flight will test both the performance of the system itself and the capability for space research. Prime targets for research will be the stratosphere and upper atmosphere, but there will also be experiments on materials processing, plasma physics, biology, astronomy and thermodynamics and lubrication. NASA and ESA next have to choose two payload specialists to work in the pressurised module; they should be selected in mid-1978.

Low-cost technology prize

The Swedish Inventors' Association is living up to its name with the announcement that it is inaugurating a highly original prize of at least $55,000 for innovations which will help to solve sociological and economic problems in the third world.

The prize will be given for their prospective, not retrospective, benefits; as it will be awarded on the hundredth anniversary of the Association's foundation, in 1986, the competition will last almost ten years. The inventors want to concentrate the competitors' efforts on certain subject-areas. The first one, announced in Stockholm recently was chosen with the firewood shortage in mind and concerns innovations related to reafforestation and the rapid production and decentralised use of wood.

Counting calories

THOMAS JUKES

A BOOK called *All Creatures Great and Small* has been on the best-seller lists for quite a while. I suppose that not all of its readers know that the verses from which the title is taken include the couplet: "The rich man in his castle, the poor man at his gate, God made them high or lowly, and ordered their estate." These lines are not best-sellers. The world is now a neighbourhood, and its inhabitants wish to shop at the same grocery as their customers whose material wants they supply. For, if manufactured products move all over the world, why should the food supply not be distributed more evenly? My tape-recorder was made in Singapore, its plug-in microphone in Japan, the cassettes in Mexico and Haiti. We grant that the race is to the swift, and the spoils to the victors, who do their best to hang on to the said spoils. However, it seems paradoxical that many people in the 'high income countries' are frantically trying to eat less, so as to live longer, and other people in impoverished lands are desperately trying to get enough food to stay alive.

Take a look at the harm done by over-eating, and at the problem of food shortages. In both cases, the central issue is calories. Too many calories lead to obesity whether they come from starch, sugar, fat or protein. Usually, however, it is carbohydrates and fats that produce obesity, with its frequent dividends of heart disease, adult diabetes and arteriosclerosis. There is a growing suspicion that risk of breast cancer and cancer of the large bowel may also be increased by high-fat diets. Obesity is not confined to inhabitants of developed countries; overeating is often one of the first responses made by people to increased availability of food in any country.

Students of diabetes have recorded that its incidence is low in populations consuming low-calorie diets, whether these are high in fat (Eskimos), high in protein (Masai) or high in carbohydrates (Indians, Zulus). Indians who move to Africa, and who then increase their caloric consumption, show a marked increase in diabetes. No longer can sleek-headed men of fat sleep soundly at night.

The diseases resulting from deprivation of food are grimmer than their counterparts in the overfed category. Calorie-deficient diets in impoverished countries are characteristically low in protein, because the cheapest sources of calories are low in protein and high in starch. Cassava meal, with a dismal 1–2% of protein, is at the bottom of the heap. It is a mainstay of many African diets. The proteins in such high-starch foods are often deficient in essential amino acids. Plants are not in business to provide animals with the good life.

The 'big three' cereals, rice, wheat, and maize, will continue to dominate the world food supply. They are deficient in lysine, tryptophan and isoleucine. Rice and wheat lack vita-A, but a million recommended daily allowances of synthetic vitamin A cost only $250. The Economic Research Service of the US Department of Agriculture, counting calories, says, "about 0.15 kilograms daily of wheat, rice, corn, sorghum, or millet would provide 500 calories. If the estimated 460 million malnourished people in the world were each provided daily with additional grain equal to 500 calories, much of the world's malnutrition would be alleviated." Where is the grain to come from? Critics of the new, high-producing strains of wheat and rice have claimed that social upheavals resulting from disruption of traditional agriculture have sometimes followed the introduction of these strains. These upheavals are accompanied by requirements for more irrigation, chemical fertilisers, and pesticides. However, for a hungry person, there is no substitute for food and, as is painfully evident in California in 1977, for a thirsty plant there is no substitute for water.

correspondence

The key to the universe

SIR,—It is always dangerous to assume that others are less intelligent than oneself. In your editorial *That Was the Weak Force, That Was* (3 February) you concede that "the initiated" found a certain amount to admire in the recent two hour programme, *The Key to the Universe*, but you add, patronisingly, "the uninitiated, which must have been 99.9% of the audience, can only have been thoroughly confused . . .". You then stand cleverly on your head and accuse the production team of one fundamental mistake: "they underestimate the serious-mindedness of the audience".

The Key to the Universe, shown on BBC2 on Thursday, 27 January, had an audience of three million; Nigel Calders' book, written in association with the programme, was soon second in the *Sunday Times* best-seller list.

We believe the public has a right to know about all aspects of science, including the esoteric, and television has a duty to inform them. It is our experience that didactic programmes attract at most half-a-million viewers: far less if made in the patronising way you advocate. I assure you that we do not underestimate the serious-mindedness of our audience and we shall continue to cater for those whom you appear to dismiss as "the uninitiated".

PHILIP DALY

Head of Science and Features,
BBC Television

Lavoisier's originality

SIR,—We have as little reason to put Lavoisier's scientific reputation on trial as had the Revolutionary Tribunal to question his loyalty to France. Gonzalez (December 9, page 504) reports that Lavoisier probably received, in 1767, a copy of John Mayow's *Opera Omnia Medico-Physica* in a shipment of 118 books ordered for his personal library.

It is much too facile to assert that "Mayow recognised the existence of oxygen, which he called *Spiritus nitro aereo*, in 1674 . . ." One might as well say that Lucretius recognised the existence of atoms 1800 years before Dalton, or Caesalpinus the circulation of blood 50 years before Harvey. If Mayow's writings were known to the 24-year-old Lavoisier in 1767, they were also known to other scientists in the preceding 93 years, yet chemistry had to wait until the 1770s for the work of Priestley, Scheele, and Lavoisier. The fact is that the idea of the *Spiritus nitro aereo* did not originate with Mayow, but derived, as Henry Guerlac has shown[1], from earlier observations concerning nitre (potassium nitrate).

As a component of gun powder, nitre was associated with explosive heat and light, but it was also associated with cold, which it produces as it is dissolved in water. An aerial spirit of nitre was therefore invoked to explain the atmospheric phenomena of thunder and lighthing, explosions so often accompanied by cold rain or hail. Since ancient times it was known that air is required for the heat of fires (for example in the use of blow pipes), and for the maintenance of animal life. Aristotle and Galen both felt that air helped to cool (to moderate) the animal body's heat. Hence in the seventeenth century an aerial spirit of nitre could be thought to provide both the heat and the coolness that the body required.

At Oxford in the years immediately preceding the young Mayow's work, Boyle and Hooke established with their vacuum pump that both a burning flame and the animal body require air; Hooke showed that the function of breathing is to supply this air rather than to squeeze by its motions the blood through the lungs, and Lower demonstrated that the blood undergoes a colour change as it passes through the aerated lungs. Much of this Mayow records in his book. He also records his own important experiments demonstrating that the combustion of certain materials, as well as respiration under certain circumstances, is accompanied by a decrease in the volume of air in an enclosed vessel. The progress of the seventeenth century scientists in understanding respiration has been lucidly summarised by Leonard Wilson[2].

Mayow was close to a discovery of oxygen in thinking that only a special part of air is involved in combustion, and that only this part is contributed by air to the blood in respiration. But his nitro-aerial spirit does not qualify as oxygen for many reasons, the most important of which are:

- ● It is not an ordinary material of definite mass or density, for Mayow asserts that it continues to exist in a vacuum, where it accounts for the conduction of light. Mayow did not claim that he had ever isolated it or measured its amount, and he did not prescribe any methods for doing so.
- ● Its qualities are too much dependent upon the circumstances impressed upon it, as for example its state of motion, which is to say that, like so much of the chemistry prior to Lavoisier, the nature of the thing was determined by too many qualifying adjectives.
- ● It is invoked to explain too wide a variety of fundamental natural phenomena, for example the nature of the sun, the presence of intense cold in the middle reaches of the atmosphere, the blueness of the sky, the nature of fire, the elasticity of air, the colours of different solid materials, and so on[3]. Incidentally, Mayow knew nothing of red blood cells and their pigment, as Gonzalez seems to imply. His explanation for the redness imparted to blood by air was that nitro-aerial particles are often of that colour, as is shown by the red distillate (nitrogen dioxide) which can be liberated by the heating of acidic nitre.

Thus it is hardly fair to assert that Lavoisier's discovery of oxygen was anticipated by Mayow, or that Lavoisier was guilty of scientific cheating in failing to give credit to him. Lavoisier's conception of oxygen was very different from Mayow's idea of the nitro-aerial spirit. And, as Guerlac has pointed out[4], Lavoisier's achievement was more fundamental even than the discovery of oxygen itself, for he was the first fully to realise that a host of material substances can exist in the gaseous state, and that the balance sheets of chemical processes must account for the materials of gases as well as of solids and liquids. Throughout his relatively short life, Lavoisier was recognised by his contemporaries as a man of exceptional genius. The day after Lavoisier was executed, the brilliant mathematician Lagrange remarked: "Only a moment to cut off that head, and a hundred years may not give us another like it"[5].

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news and views

Membrane receptor–adenylate cyclase relationships

from Melvyn F. Greaves

It is now[1] generally accepted that adenylate cyclase, through the production of cyclic AMP, plays a crucial part in the recognition and transduction of regulatory signals by cells The biological responses this enzyme helps to elicit are legion gene expression (in *E. coli*), social integration and differentiation (in slime moulds); glycogen production and fat lipolysis (in mammalian cells), catecholamine activity; and (perhaps) balding in up to half the human race. The diversity of these cellular responses raises the question of how cyclic AMP can satisfy the specificity demanded by recognition–response events.

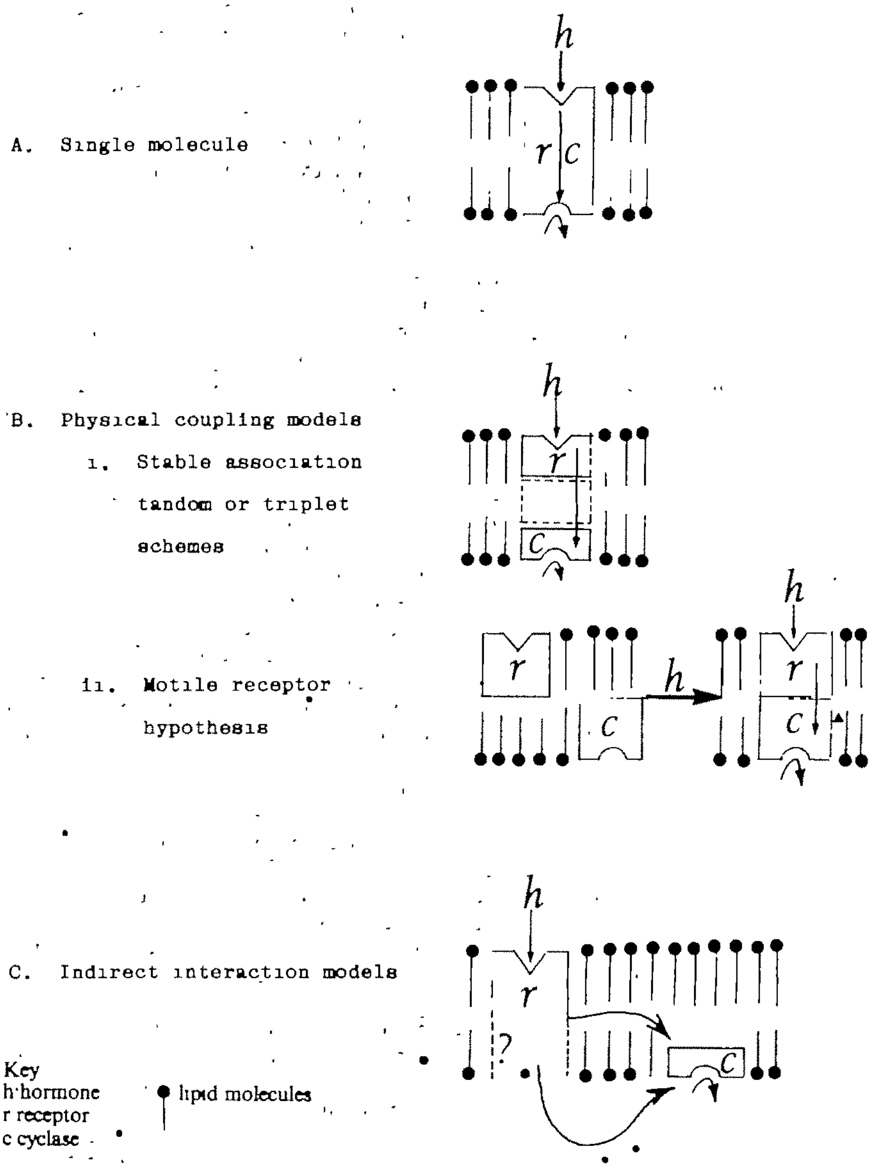
Relating adenylate cyclase activity to specialised membrane recognition events and cellular responses may not pose too great a conceptual problem with respect to mature, or 'differentiated' cells. Cyclic AMP may simply tell a cell to go faster or slower, switch on or switch off, in response to a limited number of environmental signals complementary to a restricted number of cyclase-regulating receptors. In addition, the cell's response is itself restricted qualitatively This view although simplistic is nicely supported by experiments with cholera toxin which show that stereotyped responses can be elicited by bypassing normal receptor-dependent recognition events[2]. It is also well known that cyclic AMP itself (or its dibutyryl derivative) when added to cells can also elicit the same response as the cell's normal regulators.

More serious problems are posed by the possible role of adenylate cyclase in the induction of gene programming (that is, in early 'differentiation' events) and in individual cells equipped with multiple distinct cyclase-linked hormone receptors.

A comprehensive picture of adenylate cyclase function requires much more understanding than we now have of how this enzyme's activity is integrated with other membrane and cellular events (such as ion fluxes and compartmentalisation, changes in microfilaments, membrane fluidity, activity of guanyl cyclase and phosphodiesterases and regulatory processes of gene transcription and translation

One key question is the nature of the relationship between the enzyme and receptor molecules themselves, a topic which has recently been the subject of much speculation[1-7] Figure 1 illustrates schematically the various possibilities that have been considered. It is striking that much of the debate has been dominated by the presumption that physical coupling exists between receptor and cyclase.

It is generally agreed that receptor and cyclase enzyme molecules reside mainly in opposite halves of the cell


A. Single molecule
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B. Physical coupling models
i. Stable association tandem or triplet schemes
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ii. Motile receptor hypothesis
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C. Indirect interaction models
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r receptor
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lipid molecules

Fig. 1 Models of possible physical relationships between hormone receptors and adenylate cyclase.

surface membrane with their active or binding sites orientated towards the aqueous phases respectively outside and inside the cell[8–11]. But the question of how they communicate remains. Are their 'ends' in contact with lipid as a lubricant or have they no physical continuity or proximity at all? What form does the modulation of cyclase activity take?

Following their extensive studies of adenylate cyclase regulation by the hormone glucagon and guanine nucleotides, Rodbell and colleagues[12,13] have suggested some answers to the last question (see also a recent review by Helmreich *et al.*[5]). They postulate that adenylate cyclase exists in three interconvertible states differing in catalytic activity and susceptibility to binding site inhibition by protonated substrate. Hormone–receptor interaction is thought to augment the normally slow (and spontaneous) transition of cyclase to that catalytic state in which it is least susceptible to inhibitory substrate. Binding of guanyl nucleotides to a regulatory site is thought to have a crucial intermediary or 'transducer' role in this hormone-facilitated transition. The favoured mechanism here, as in some other receptor systems, is allosteric interaction. Receptors are envisaged as regulatory molecules capable of exercising influence over alternative physical conformations of a partner 'effector' molecule (the cyclase enzyme or an intermediate). Hormone–receptor complexes then facilitate or stabilise that conformation of effector most commensurate with the appropriate biological response to the initiating ligand

A 'mobile receptor' hypothesis for receptor–cyclase coupling has been proposed by Cuatrecasas[4,14,15] who suggests that hormone-induced clustering of membrane receptors within the membrane facilitates contact and interaction with cyclase enzymes. This model derives its impetus from observations on lateral redistribution of exposed cell surface antigens (by antibodies), glycoproteins (by lectins) and gangliosides (by cholera toxin)[16]. A similar theoretical model has also been proposed recently by McGuire and Barber[17].

The capacity of a particular receptor to be redistributed (by a hormone, for example) will depend, amongst other things, on receptor density, hormone concentration, the valency of interactions, the association of receptor with other molecules (such as lipids of varying degree of saturation and fluidity and submembrane microfilaments) and the overall organisational symmetry of the cell in terms of its orientation, association with neighbours and ordered non-random structure of its membrane. Binding studies with labelled insulin suggest that receptors for this hormone may exist as small clusters[4,14]; however there is as yet no convincing demonstration of hormone-induced receptor clustering and there may be situations where the required redistribution is impossible

. Cuatrecasas[14] cites the instance of morphologically-polarised epithelial cells (in gut, liver and bladder for example), in which hormone receptors and adenylate cyclase may normally be concentrated at opposite sides of the cell. He argues that this situation is quite incompatible with any form of permanent structural coupling and suggests that direct interaction between receptor and enzyme could be brought about by redistribution of the mobile hormone receptor. This mechanism requires the hormone–receptor complex to undertake a rather extensive journey around the sides of the cell towards the 'distal' pole (see Fig 23 in ref. 14).

Although such large scale polarised redistribution is seen, and was of course discovered, in lymphocytes, these are motile cells with no intrinsic polarisation of receptors. Whatever mechanism of large scale redistribution is favoured[16,18], it seems rather unlikely that such membrane behaviour would be possible in non-motile tissue cells bound by various intercellular junctions. Indeed if the strict polarisation of receptors and adenylate cyclase is correct this situation provides a powerful argument against all models of receptor–cyclase interaction favouring physical coupling.

Alternative models which demand no physical coupling between receptor and cyclase enzyme must therefore be considered. Perkins[2], for example, has proposed that hormone receptors may modulate cyclase enzyme activity indirectly through a lipid 'field' effect of undefined nature.

An ingenious technique which may shed some light on receptor–cyclase relationships has been used recently by Orly and Schramm[20]. These authors (using Sendai virus) fused turkey erythrocytes, in which the endogenous beta adrenergic receptor had been inactivated by heat or N-ethylmaleimide, with Friend erythroleukaemia cells The latter have adenylate cyclase but no demonstrable beta adrenergic receptor The objective of the fusion experiment was then to 'couple' the turkey erythrocytes' beta adrenergic receptor with the cyclase derived from the leukaemic cells. Within a few minutes of fusion adenylate cyclase activity (measured by cyclic AMP formation by cell ghosts) could indeed be considerably enhanced by isoproterenol. The levels of activity recorded with a single concentration of isoproterenol (50 μM) were at least as high in the 'coupled hybrid' as in the untreated turkey erythrocyte cells incubated with isoproterenol or sodium fluoride.

This system promises to be an exciting new way of investigating lesions in membrane receptor function. Does it tell us anything about the receptor–cyclase linkage itself? Orly and Schramm interpret their results in terms of the model developed principally by Rodbell and colleagues in which the hormone receptor interacts with cyclase by way of a guanyl nucleotide binding component. However, this still leaves open the question of the physical relationship of hormone binding site, guanyl nucleotide binding site and cyclase catalytic site. One broad conclusion which seems readily available from the fusion experiments is that whatever the physical receptor–cyclase relationship, it is established with remarkable speed and apparent ease. More specifically the experiment implies that the beta adrenergic receptor is not normally coupled to an endogenous cyclase which is sub-type specific or peculiar to this receptor. This would appear to accord with the earlier studies of Rodbell[6,21] and others[3,22] who demonstrated that several distinct hormone receptors appeared to 'compete' or have access to a common pool of adenylate cyclase If correct these interpretations weaken any direct coupling models. Several other factors may also argue against a direct interaction of receptor and cyclase. Binding of some hormones (growth hormone for instance) to exceedingly few receptors, constituting a minute fraction of the cell surface area, can elicit within seconds dramatic effects of cell membrane activity such as conformational changes detected by spectroscopic methods and ion fluxes[7].

Taking into consideration the efficacy of such small numbers of receptor molecules along with the ease and speed of functional receptor–cyclase recoupling in the hybrid experiments it seems very likely that some rapid amplification step occurs between hormone receptor molecules and adenylate cyclase modulation.

Sonnenberg and Schneider have recently proposed a comprehensive model for hormone action drawing heavily on analogy with neurotransmitters[7]. They suggest that multiple membrane-associated events involving enzyme activation and microfilament changes may follow, be dependent on, and be propagated by, rapid alterations in ion fluxes and electric potential initiated by a small number of receptors. For this model to be plausible there is no reason to suppose the cyclase enzyme has, or requires, physical contact with any part of the hormone receptor molecule. This model, like its predecessors, is a working hypothesis requiring verification or denial by experiment As it stands the idea has a seductive and perhaps illusory simplicity. If its basic thesis is correct then it may in fact be even more difficult to unravel the circuitry and causal relationships involved in membrane signal transduction processes than was earlier supposed. ☐

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Breakdown of superfluidity in liquid helium

from A. J. Leggett

IT has been known for more than 60 years that liquid helium (^{4}He) is superfluid below about 2 K; that is, it flows through narrow capillaries without observable friction, and, conversely, an object moving slowly through ^{4}He at low temperatures experiences practically no friction either. A major step forward in understanding this phenomenon was made by the Russian physicist Lev Landau in 1941; he showed that superfluidity is a natural consequence of certain assumptions about the 'elementary excitations' of the liquid, and predicted the critical velocity above which normal behaviour should set in. In a recent paper (*Phil. Trans R Soc.* **284**, 179; 1977) D. R. Allum *et al.* report the first unambiguous observation of this 'Landau critical velocity', thereby strongly confirming Landau's ideas. They also report some surprising and significant features of the mechanism by which superfluidity breaks down above the critical velocity.

An 'elementary excitation' is the simplest (quantised) disturbance which can be created in a given system; generally, it will carry a momentum p and an energy E which depends on p. Landau argued that at sufficiently low temperatures a heavy external body travelling through the system can experience friction only if it can create elementary excitations. However, conservation of momentum and energy prevent this happening at velocities less than the Landau critical velocity v_L given by

$$v_L = \min(E(p)/p)$$

A slight modification of the argument gives the same critical velocity for helium flowing through a capillary. In the case of a normal classical liquid it is easy to show that v_L is zero, so there is no superfluidity.

For liquid helium below 2 K, however, it is believed that the elementary excitation spectrum (the graph of $E(p)$ against p) should have the form shown in the figure. The excitations of small p (long wavelength) are quantised sound waves, or phonons, whereas those near the minimum of the curve are called 'rotons'. Originally a roton was envisaged as a quantised rotational motion of the liquid, but more recent theories have suggested some modifications to this picture and its physical nature is still somewhat obscure. The existence of rotons and more generally the correctness of the $E(p)$ curve of the figure has been confirmed both by specific-heat data and by experiments in which a neutron is scattered from the liquid by creating a single elementary excitation.

It is obvious from the figure that the minimum of $E(p)/p$ occurs near the roton minimum in the curve and is in fact given by the slope of the straight line; numerically, this corresponds to a value of v_L of about 50 m s^{-1}. At velocities higher than this emission of one or more rotons will occur, giving rise to friction. However, experiments on the flow of helium through narrow capillaries usually yield much lower (and often geometry-dependent and/or irreproducible) critical velocities, probably owing to the existence of tangles of vorticity in the liquid.

Allum and coworkers used the alternative technique of measuring the frictional drag on bodies moving through the liquid—in this case, negative ions. (A negative ion in liquid helium is believed to be a complex object composed of an electron plus the cavity it excavates for itself in the liquid and the portion of the helium which moves with it; the whole has a diameter of the order of 10 Å and a mass of about 70 helium-atom masses). When plotted as a function of electric field E, the mean velocity of the ions, $\bar{v}$, rises virtually instantaneously from zero to a value which is of order 50 m s^{-1} and coincides within the experimental error with the value of v_L predicted from the excitation spectrum. This indicates strongly that at velocities less than v_L there is indeed no frictional drag, in accordance with Landau's ideas.

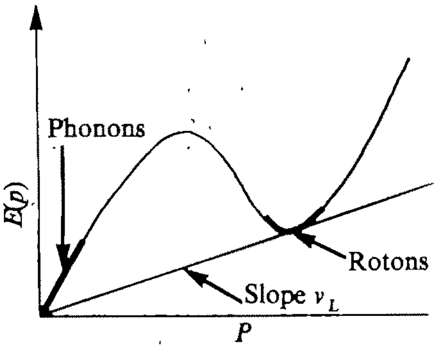


Fig. 1 Elementary excitation spectrum of superfluid helium.

A second interesting and quite unexpected result emerges from a comparison of the observed behaviour of $\bar{v}$ as a function of E with a recent theory, due to R. M. Bowley and F. W. Sheard, of the frictional drag at velocities above v_L. They showed that if the dominant process by which the ion loses energy and momentum is creation of single rotons, the quantity $v - v_L$ should be proportional to $E^{3/4}$, whereas if it is creation of pairs of rotons which dominates it should be proportional to $E^{1/3}$. For most of the range of fields studied the data fit an $E^{1/3}$ dependent; they cannot possibly be reconciled with the $E^{3/4}$ one. Thus, it seems that negative ions moving in helium create rotons only, or at least predominantly, in pairs whereas neutrons seem to have no difficulty in creating them singly! This intriguing result seems certain to stimulate further research, both experimental and theoretical, into the physical nature of rotons. ☐

Dynamic Stark splitting

from Peter Knight

WORKERS in fields other than quantum optics or atomic physics may be surprised to hear that resonance fluorescence, that classic phenomenon of early quantum physics, has shrugged off its antiquarian associations to become a major and intensively studied new research problem. The rejuvenating influence has been the tunable laser. Unlike life, 'undressed' atoms, those old-fashioned atoms bathed in a weak radiation field, become very much more interesting when 'dressed' by an intense resonant radiation field. The interest has shifted from a purely spectroscopic analysis of atomic structure to the basic mechanisms by which any atom interacts with radiation.

At low excitation intensities the process of resonance fluorescence is well understood: if the excitation field is monochromatic, the atom absorbs a photon at the excitation frequency, and energy conservation demands that the emitted photon have the same frequency. One-photon processes are dominant. The emitted fluorescence has the same spectral profile, and in particular the same narrow width, as the excitation. Resonance fluorescence with a spectral width narrower than the natural linewidth has been observed for the first time, by Gibbs and Venkatesan at Bell Laboratories (*Opt. Commun.* **17**, 87; 1976), using the dye-laser excited 2,852 Å ^{24}Mg transition, and by Wu, Grove and Ezekiel at the Massachusetts Institute of Technology (*Phys. Rev. Lett.* **35,** 1426; 1975), using the 5,890 Å D line of sodium.

Of more interest is what happens to the fluorescence process as the laser intensity increases. In a strong resonant field, non-linear scattering occurs. Multiphoton processes become as important as single photon processes. Energy conservation holds overall for the multiphoton process, but the fluorescence spectrum is no longer monochromatic. The atom can coherently interact many times with the laser field before spontaneously radiating a photon. Clearly we can no longer rely on the conventional low-order perturbation theory of resonance fluorescence.

An atom of principal transition frequency ω_0 interacting with a coherent laser field of frequency ω is stimulated to emit and reabsorb photons. The rate at which transitions are coherently induced between the two atomic levels is known as the Rabi frequency and is proportional to the square root of the laser intensity. One can think of the atom heuristically as a damped driven quantum oscillator, the damping being supplied by spontaneous emission and the driving force by the interaction with the laser field. At low intensities, spontaneous emission is more important than stimulated processes, and the atom behaves as an over-damped oscillator, radiating a field centred upon the driving frequency. At higher intensities the stimulated Rabi nutational frequency can exceed the spontaneous emission rate and the atom behaves just like an under-damped oscillator. The Rabi oscillations show up not only in the population of the atomic levels (the inversion) but also as a modulation of the quantum dipole moment. This in turn leads to sidebands emerging in the spectrum of the emitted radiation, and this so-called "dynamic Stark splitting" has attracted a great deal of interest.

The basic theory to describe monochromatic strong-field resonance fluorescence was developed by Mollow some time ago (*Phys. Rev.* **188,** 1969; 1969). Mollow calculates the emission spectrum directly from the Fourier transform of the atomic dipole moment correlation function. This correlation function (if steady state is assumed) can be expressed in terms of the atomic inversion and dipole moment produced by the strong laser field interaction. Qualitatively we expect to find a strong-field fluorescence spectrum with a central spontaneous emission line, and two subsidiary sidebands separated from the central component by the Rabi frequency. Mollow's theory demonstrates just how this happens in detail and predicts a spectrum made up of three Lorentzian-shaped lines; a strong central component at the laser frequency and two sidebands with peak intensities 1/3 that of the central line (see Fig. 1). The central component has the natural width Γ. The sidebands have a width 3Γ/2 and are displaced from the central peak by the Rabi frequency Ω, which is proportional to the amplitude of the laser electric field strength. Many authors have contributed to the detailed understanding of the fluorescence spectrum, but the broad features are all contained in the Mollow theory.

Experimental work by three groups all using dye-laser excited atomic beam methods broadly confirm Mollow's original prediction. Schuda, Stroud and Hercher (*J. Phys.* **B7,** L198; 1974), Ezekiel *et al.* (Wu, Grove & Ezekiel (*op. cit.*), and Walther and coworkers, at Köln and München (*Z. Physik.* **A278,** 205; 1976) have all observed the triplet splitting as the intensity increases. Certain asymmetries of the lineshape, almost certainly due to optical pumping effects extraneous to the basic phenomenon, have been noted and in part eliminated by careful preparation of the atoms by circularly polarised light.

Theoretical interest seems now to be concentrating on the role of the state of coherence of the incident laser radiation in these basically multiphoton processes. Intensity fluctuations will obviously be important as a 'smearing-out' effect: the a.c. Stark splitting is proportional to the square root of the instantaneous intensity. Similarly, phase fluctuations will create jitter in atomic dipole moments and correlation functions will be additionally damped. To analyse the effects of a partially coherent field, and to generalise the purely monochromatic field approach of Mollow is not easy. The detailed dynamics of an atom interacting with an intense field are described by the optical Bloch equations (analogous to nuclear magnetic resonance equations of motion). These equations can be solved straightforwardly by a Laplace transform technique if the radiation field is purely monochromatic, but this is not directly applicable if the field is partially coherent. Neither do traditional Bloch–Wangsness relaxation methods apply, because the laser power is too high.

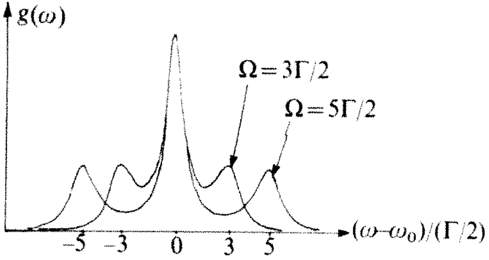
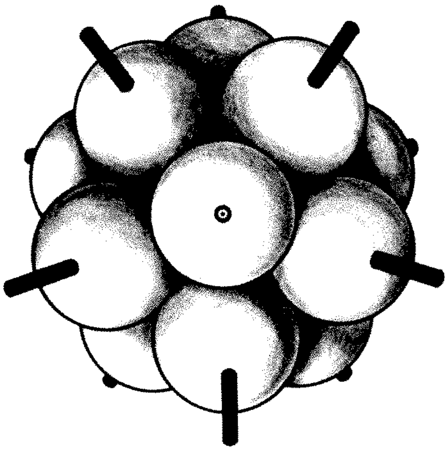


Fig. 1 The emission spectrum $g(\omega)$ for a resonantly driven two-level atom for two different laser intensities according to Mollow's theory.

Recently, a non-perturbative theory of atomic relaxation in the presence of intense partially coherent radiation has been developed by Eberly (*Phys. Rev. Lett.* **37,** 1387; 1976) and applied to the theory of resonance fluorescence. By including stochastic phase and amplitude fluctuations of the laser directly in the equations of motion for the atomic dipole and inversion operators and assuming a simple exponential damping of first order phase and amplitude correlations, he describes how the Mollow theory of resonance fluorescence is modified by the finite bandwidth of the laser field. He is also able to include the effects of the interferometer analysing the radiated field spectrum. His results indicate that laser phase and amplitude fluctuations can alter the shape of the fluorescence spectrum in an unexpected and non-additive way. Amplitude fluctuations increase the height of the centre peak relative to the sidebands; phase fluctuations lower it. Eberly's results have been confirmed by Agarwal (*Phys. Rev. Lett.* **37,** 1383; 1976) using much more complex and physically less transparent methods. Agarwal goes on to look at the effect of laser bandwidth on the statistics of the emitted radiation. It is known from the work of Walls and others (Carmichael & Walls *J. Phys.* **B9,** 1199; 1976) that the emitted radiation should exhibit a fundamentally quantum feature called "antibunching", in contrast to the photon bunching familiar from the Hanbury-Brown Twiss experiment. Agarwal's results indicate that this too is affected by the laser bandwidth. ☐

ΦX174 sequenced



from Maria Szekely

A GREAT advance in DNA research is reported in this issue: the first complete nucleotide sequence of a DNA genome has been established by Sanger's group in Cambridge (see page 687 of this issue of *Nature*). For the first time it is possible to look at a viral genome as a giant molecule of known structure, all functions of which should be accessible to interpretation in terms of its molecular structure.

The small coliphage ΦX174 is particularly suitable for studies on the structural features underlying the complex processes of gene expression. It has a number of unusual characteristics—its small DNA possesses an unexpectedly high information content and seems to exploit every structural possibility to control gene expression. The single-stranded circular DNA genome of 5,375 nucleotides codes for nine proteins, some of which have been directly sequenced. The amino acid sequence of the others can be deduced from nucleotide sequence data. Genetic and physical maps of the genome have been constructed and have been correlated recently by Weisbeek *et al.* (*Virology* **72,** 61; 1976) who estimated the probable length of each gene. But there is a discrepancy between the calculated coding potential of some regions in the physical map and the proteins produced. Replication and transcription of the genome have also been studied extensively, with some unexpected results. Baas *et al* (*J. molec. Biol.* **102,** 633; 1976) located the origin of replication in the middle of a structural gene coding for protein A. Hayashi *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **73,** 3519; 1976) reported the presence of two mRNAs covering the *A* gene region, only one of which was functionally active. The complete sequence has now provided a sound structural basis for these peculiarities.

ΦX174 DNA has been the subject of nucleotide sequence studies from early on. Practically all DNA sequencing techniques have been applied to this molecule, and some have been actually worked out on this DNA. Five years ago classical degradation techniques only allowed the determination of sequences of about 50 nucleotides which made the task of resolving the primary structure of DNA molecules seem much more difficult than the sequencing of RNA. Introduction of new techniques, making use of primed synthesis of highly-labelled complementary strands on ΦX DNA template greatly extended these possibilities. Eventually the new highly efficient 'plus and minus' technique of Sanger and Coulson (*J. molec. Biol.* **94,** 441; 1975) which allows 150–200 nucleotide long sequences to be read directly, made it possible to produce a complete sequence of the ΦX genome a year after the first (and so far only) complete sequence of an RNA genome had been determined (Fiers *et al. Nature* **260,** 500; 1976).

Together with these sequence determinations, information on characteristic structural features of some functional sites of the DNA has also contributed to the construction of a 'chemical map' of the ΦX genome. 5′-terminal sequences of three mRNAs produced *in vitro* have been determined by Smith and Sinsheimer (*J. molec. Biol.* **103,** 699 and 711; 1976); ribosome binding sites on mRNAs corresponding to the initiation of genes *B*, *J*, *F* and *G*, have been sequenced by Ravetch *et al.* (page 698 of this issue). Homologies with other promoter and terminator sequences as well as the presence of purine tracts at all ribosome recognition sites helped to locate each gene in the sequence. At the same time, these homologies confirmed that the structure suggested for these control signals is generally valid.

The interesting question now arises of how the peculiar properties of ΦX can be interpreted in the knowledge of the primary structure. The most intriguing aspect is how the very high information content can be compressed into a small DNA molecule.

It now seems that this phage has several ways of ensuring the most economical use of its DNA. The most spectacular discovery has already been reported—the same stretch of DNA can code for two proteins which are translated in different reading frames (*News and Views* **264,** 11; 1976). In 1976 Barrell *et al.* (*Nature* **264,** 34) found that the complete *E* gene was contained within the *D* gene region. A similar situation is reported now for genes *B* and *A*. Two papers in this issue provide evidence that gene *B* is contained within the region of gene *A*: Brown and Smith (page 695) locate the ribosome binding site for gene *B* within the gene *A* region, and Smith *et al.* (page 702) assign mutants in gene *A* and gene *B* to different reading frames in the overlapping area.

Another DNA stretch which fulfils multiple functions was found at the beginning of the *A* gene, where the 3′-terminal part of an mRNA which had started at an earlier promoter overlaps with the beginning of the mRNA for the *A* gene. The sequence of this DNA stretch suggests that a specific hairpin loop structure may be involved in the control mechanism which provides within this overlap a termination signal for one mRNA without interfering with the initiation of transcription of another mRNA. Readthrough at the termination signal may explain Hayashi's finding of two mRNAs covering this area. Two more overlaps occur at the initiation sites of proteins C and J, where the initiation codons overlap with the termination codons of the previous cistrons. The purine tracts preceding the initiation codon serve thus both as recognition sites for ribosomes and as coding sequences for another protein. Gene *A* seems the most versatile part of the genome: in addition to the multiple functions listed above, it also contains the origin of replication, and thus recognition sites for the proteins involved. The exact site of the origin has not been located, but the region in gene *A* which contains this site has been defined. Its sequence is less characteristic than could have been anticipated, it shows no symmetry or secondary structure, only a probable specific arrangement of an A–T rich stretch between the G–C rich stretches.

The multifunctional character of ΦX DNA may be a unique property displayed only by a few very small DNA viruses. Overlapping termination and initiation codons have also been detected, however, in the tryptophan messenger of *Escherichia coli* (Platt & Yanofski *Proc. natn. Acad. Sci. U.S.A.* **72,** 2399; 1975), the possibility thus exists that at least some of the above ways to compress more information into DNA may be of more general significance. If this were so, our ideas about the coding potential of DNA genomes would have to be fundamentally altered.

X-ray laser quest

from I. J. Spalding

For the alchemists it was the Philosopher's Stone, for the fusion specialist it's 'scientific break-even', but for some laser enthusiasts their chosen—and one hopes attainable—goal is the X-ray laser. The advent of high brightness, uniquely monochromatic, coherent light sources in the visible and infrared regions of the electromagnetic spectrum has already completely transformed many interferometric, scattering and spectroscopic techniques, and has opened up prospects for entirely novel applications. One might expect that the development of coherent X-ray sources would similarly revolutionise such tasks as high-resolution, time-resolved X-ray diagnostics and crystal structure determination.

Why is this goal difficult to achieve? One fundamental problem lies in the short life time of the upper levels of interest: the spontaneous life time for a 10 keV K–L transition in copper metal might typically be 10^{-15} s, implying the gargantuan pumping requirement of ~1 watt per atom! Several ingenious schemes have been proposed to provide such high pumping rates. These include Gudzenko and Shelepin's proposal for rapid recombination in a dense (laser-produced) plasma, Duguay and Rentzepis' photoionisation scheme, and McCorkle's suggestion for sweeping a high-current ion beam at the speed of light along an obliquely-oriented foil target.

Not surprisingly, such novel physical conditions may pose unusual theoretical problems. Bonifacio, Hopf, Meystre and Scully have recently reviewed quantum electrodynamic modifications to conventional small-signal gain theory which become necessary in a travelling wave X-ray laser where the pump behaves as a delta function swept at the velocity of light along the active medium. Depending on how fast the population inversion decays compared with the atomic polarisation, the amplification may be substantially smaller than that anticipated from semi-classical (Maxwell–Schrödinger) theory. Happily, large-signal pulse amplification becomes more favourable in the non-linear regime since coherence brightening, super radiant effects and power broadening (that is, broadening of energy levels due to the e.m. electric field) are predicted to reduce saturation effects.

Most X-ray laser experimenters have concentrated their attention on transitions in laser-generated plasmas at wavelengths in the extreme ultraviolet, where detailed numerical computations have shown that reasonable gains are achieveable using the high power lasers now available. The plasma conditions and computer codes required are similar to those of interest in laser fusion. In 1974 Irons and Peacock reported population inversion between states of principal quantum number $n=2$–5 in an expanding low-density C^{+5} laser-produced plasma, and recently Dewhurst *et al.* (*Phys. Rev. Lett.* **37**, 1265; 1976) have demonstrated inversion of the $n=3$ to $n=2$ transition of CVI at 182 Å at densities which it is claimed are sufficiently high to permit (length) scaling to useful gains.

This approach relies on an extremely rapid expansion and cooling of a fully ionised plasma; recombination then leads to a preferential population of the upper states, and hence an inversion. In the work of Dewhurst *et al.* the target was a 5.3-μm diameter carbon fibre supported vertically at the focus of a 0.15 J Nd–glass laser pulse of duration 1.4×10^{-10} s. The fibre was first shattered by a carefully chosen prepulse, and then irradiated by the main pulse having a contrast ratio exceeding 10^4. The width of the main pulse was determined by the time needed to heat and ionise the dense cold plasma formed by the prepulse. The presence of inversion was deduced from the relative intensities of (time averaged) Lyman series lines, photographically recorded by an absolutely calibrated 2 m grazing incidence spectrograph. Space-averaged spectra could be obtained using a single laser shot, but an average of 30 exposures was required when the radial resolution was narrowed to 100 μm. The radial variation of L_α, L_β, and L_λ, intensities accords well with their numerical model, and thus they compute a maximum gain-length product of 2×10^{-2} for their experiment. They estimate that a 100 J laser should permit a gain-length product of ~10, and so permit laser operation in a travelling-wave mode, and that the gain should vary as $Z^{7.5}$, where Z is the charge number of the ion. However for $Z\geqslant13$ laser compression would be needed to achieve the very high densities (and small radii) required.

Although these and other developments are encouraging it should be noted in conclusion that it seems unlikely that regenerative feedback in the extreme ultraviolet will be possible, and that the shortest wavelength coherent sources are currently produced by non-linear optical conversion in solids and gases. (The record is temporarily held by a group at the US Naval Research Laboratory, who have in this way generated 380 Å, the 28th harmonic of the Nd–YAG 1.06 μm laser.) □

Transform faults

from Peter J. Smith

When Wilson (*Nature* **207**, 343; 1965) originally defined transform faults, he suggested that the transform faults offsetting the oceanic ridges were directly inherited from the pattern of continental rifting which had led to the formation of the relevant plate boundary. But many Earth scientists have never been entirely happy with this view of the origin of ridge-to-ridge faulting. Nor have they been happy with the lumping together of all transform faults into a single class—a class which fails to distinguish between, for example, the long San Andreas fault and the shortest of faults offsetting the mid-Atlantic ridge.

A distinction is now made by Gilliland and Meyer (*Geol. Soc. Am. Bull.* **87**, 1127; 1976) who divide transform faults into 'boundary transform faults' and 'ridge transform faults'. Boundary transform faults are major faults genuinely inherited from the original rifting which gives rise to a plate and "are necessary initially to permit differential movement between discrete plates". They are thus contemporaneous with the formation of the plate. They are most likely to be ridge-to-trench faults and least likely to be ridge-to-ridge, although the latter are not excluded. Examples of boundary transform faults quoted include the Alpine, Denali, New Guinea, Philippines and San Andreas faults and the Ninetyeast ridge.

By contrast, ridge transform faults occur only along oceanic ridges and are thus always ridge-to-ridge types. They are not inherited from initial rifting but (as wax models show) result from the spreading process; that is, they are contingent upon the existence of spreading. They are secondary features and thus not generally contemporaneous with plate formation. Indeed, in theory they are not necessary for plate boundary formation at all and do not always exist when spreading rates are very low.

A weak point in the argument seems to arise from the need to admit ridge-to-ridge faults into both major categories. For example, Gilliland and Meyer regard the San Andreas fault as a boundary transform fault, although they concede that "its real nature is not by any means certain". Apart from the size of this particular fault and the fact that it impinges on land, the precise grounds for distinguishing it from ridge-to-ridge faults in the ridge transform fault category are not made entirely clear.

articles

Nucleotide sequence of bacteriophage ΦX174 DNA

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A DNA sequence for the genome of bacteriophage ΦX174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus and minus' method. The sequence identifies many of the features responsible for the production of the proteins of the nine known genes of the organism, including initiation and termination sites for the proteins and RNAs. Two pairs of genes are coded by the same region of DNA using different reading frames.

THE genome of bacteriophage ΦX174 is a single-stranded, circular DNA of approximately 5,400 nucleotides coding for nine known proteins. The order of these genes, as determined by genetic techniques[2–4], is *A–B–C–D–E–J–F–G–H*. Genes *F*, *G* and *H* code for structural proteins of the virus capsid, and gene *J* (as defined by sequence work) codes for a small basic protein that is also part of the virion. Gene *A* is required for double-stranded DNA replication and single-strand synthesis. Genes *B*, *C* and *D* are involved in the production of viral single-stranded DNA: however, the exact function of these gene products is not clear as they may either be involved directly in DNA synthesis or be required for DNA packaging, which is coupled with single-strand production. Gene *E* is responsible for lysis of the host.

The first nucleotide sequences established in ΦX were pyrimidine tracts[5–7] obtained by the Burton and Petersen[8] depurination procedure. The longer tracts could be obtained pure and sequences of up to 10 nucleotides were obtained. More recently Chadwell[9] has improved the hydrazinolysis method to obtain the longer purine tracts. These results are included in the sequence given in Fig. 1.

More extensive ΦX sequences were obtained using partial degradation techniques, particularly with endonuclease IV (refs 10 and 11). Ziff *et al.*[12,13] used this enzyme in conditions of partial hydrolysis to obtain fragments 50–200 nucleotides long which were purified as ^{32}P-labelled material by electrophoresis on polyacrylamide gels. The fragments came from the same region of the genome and the sequence of a 48-nucleotide long fragment (band 6, positions 1,047–1,094) was determined using mainly further degradation with endonuclease IV and partial exonuclease digestions.

Another 50-nucleotide long fragment was obtained by Robertson *et al.*[14] as a ribosome binding site. The viral (or plus) strand DNA of ΦX has the same sequence as the mRNA and, in certain conditions, will bind ribosomes so that a protected fragment can be isolated and sequenced. Only one major site was found. By comparison with the amino acid sequence data it was found that this ribosome binding site sequence coded for the initiation of the gene *G* protein[15] (positions 2,362–2,413).

At this stage sequencing techniques using primed synthesis with DNA polymerase were being developed[16] and Schott[17] synthesised a decanucleotide with a sequence complementary to part of the ribosome binding site. This was used to prime into the intercistronic region between the *F* and *G* genes, using DNA polymerase and ^{32}P-labelled triphosphates[18]. The ribo-substitution technique[16] facilitated the sequence determination of the labelled DNA produced. This decanucleotide-primed system was also used to develop the plus and minus method[1]. Suitable synthetic primers are, however, difficult to prepare and as DNA fragments generated by restriction enzymes are more readily available these have been used for most of the work reported here.

Another approach to DNA sequencing is to make an RNA copy using RNA polymerase with α-^{32}P-labelled ribotriphosphates and then to determine the RNA sequence by more established methods. Blackburn[19,20] used this approach on intact single-stranded ΦX and on fragments obtained by digestion with endonuclease IV or with restriction enzymes. Sedat *et al.*[21] were extending their studies on the larger endonuclease IV fragments and their results, taken in conjunction with the transcription of the DNA fragments[20], amino acid sequence of the F protein[22], and the plus and minus method results, made it possible to deduce a sequence of 281 nucleotides (positions 1,016–1,296, Fig. 1) within the *F* gene[23]. Transcription of *Hin*dII fragment 10, amino acid sequence data in the *G* gene, and the plus and minus method using *Hin*dII fragments 2 and 10 as primers, gave a sequence of 195 nucleotides (positions 2,387–2,582, Fig. 1) at the N terminus of gene *G* (ref. 24).

The 'plus and minus' method

Further work on the ΦX sequence has been done using chiefly the plus and minus method primed with restriction fragments. Figure 2 shows the various restriction enzymes used and the fragment maps for each (refs 25–30 and C.A.H., submitted for publication, and N.L.B., C.A.H. and M.S., submitted for publication).

Figure 1 shows the combined results of the sequence work to date. The sequence is numbered from the single cleavage site of the restriction enzyme *Pst*I. As with other methods of sequencing nucleic acids, the plus and minus technique used by itself cannot be regarded as a completely reliable system and occasional errors may occur. Such errors and uncertainties can only

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P1/1 R5/7b F6/9 T7/8 T8/9
GAGTTTTATC GCTTCCATGA CGCAGAAGTT AACACTTTCG GATATTTCTG ATGAGTCGAA AAATTATCTT GATAAAGCAG GAATTACTAC TGCTTGTTTA CGAATTAAAT CGAAGTGGAC
10 20 30 40 50 60 70 80 90 100 110 120
End B ↑

A5/18
T9/10 H8b/4 T10/4 A18/6 F9/13
TGCTGGCGGA AAATGAGAAA ATTCGACCTA TCCTTGCGCA GCTCGAGAAG CTCTTACTTT GCGACCTTTC GCCATCAACT AACGATTCTG TCAAAAACTG ACGCGTTGGA TGAGGAGAAG
130 140 150 160 170 180 190 200 210 220 230 240
End A ↑

F13/17 F17/16a R7b/6c F16a/16b
TGGCTTAATA TGCTTGGCAC GTTCGTCAAG GACTGGTTTA GATATGAGTC ACATTTTGTT CATGGTAGAG ATTCTCTTGT TGACATTTTA AAAGAGCGTG GATTACTATC TGAGTCCGAT
250 260 270 280 290 300 310 320 330 340 350 360
mRNA start ↑

F16b/1 Z3/7 A6/1
GCTGTTCAAC CACTAATAGG TAAGAAATCA TGAGTCAAGT TACTGAACAA TCCGTACGTT TCCAGACCGC TTTGGCCTCT ATTAAGCTCA TTCAGGCTTC TGCCGTTTTG GATTTAACCG
370 380 390 400 410 420 430 440 450 460 470 480
D start ↑

M1/7 T4/5
AAGATGATTT CGATTTTCTG ACGAGTAACA AAGTTTGGAT TGCTACTGAC CGCTCTCGTG CTCGTCGCTG CGTTGAGGCT TGCGTTTATG GTACGCTGGA CTTTGTAGGA TACCCTCGCT
490 500 510 520 530 540 550 560 570 580 590 600
E start ↑

R6c/7a Z7/5 H4/13
TTCCTGCTCC TGTTGAGTTT ATTGCTGCCG TCATTGCTTA TTATGTTCAT CCCGTCAACA TTCAAACGGC CTGTCTCATC ATGGAAGGCG CTGAATTTAC GGAAAACATT ATTAATGGCG
610 620 630 640 650 660 670 680 690 700 710 720

T5/3 Y1/3 H13/11 M7/3
TCGAGCGTCC GGTTAAAGCC GCTGAATTGT TCGCGTTTAC CTTGCGTGTA CGCGCAGGAA ACACTGACGT TCTTACTGAC GCAGAAGAAA ACGTGCGTCA AAAATTACGT GCGGAAGGAG
730 740 750 760 770 780 790 800 810 820 830 840
End E ↑

H11/14 H14/12 R7a/6b
TGATCTAATG TCTAAAGGTA AAAAACGTTC TGGCGCTCGC CCTGGTCGTC CGCAGCCGTT GCGAGGTACT AAAGGCAAGC GTAAAGGCGC TCGTCTTTGG TATGTAGGTG GTCAACAATT
850 860 870 880 890 900 910 920 930 940 950 960
End D ↑ ↑ J start

Z5/8 H12/10
TTAATTGCAG GGGCTTCGGC CCCTTACTTG AGGATAAATT ATGTCTAATA TTCAAACTGG CGCCGAGCGT ATGCCGCATG ACCTTTCCCA TCTTGGCTTC CTTGCTGGTC AGATTGGTCG
970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
↑ End J F start ↑

Y3/2 F1/14b T3/1 H10/7 Z8/4 F14b/2
TCTTATTACC ATTTCAACTA CTCCGGTTAT CGCTGGCGAC TCCTTCGAGA TGGACGCCGT TGGCGCTCTC CGTCTTTCTC CATTGCGTCG TGGCCTTGCT ATTGACTCTA CTGTAGACAT
1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200

Q1/3c R6b/1
TTTTACTTTT TATGTCCCTC ATCGTCACGT TTATGGTGAA CAGTGGATTA AGTTCATGAA GGATGGTGTT AATGCCACTC CTCTCCCGAC TGTTAACCAA ACTACTGGTT ATATTGACCA
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320

H7/5
TGCCGCTTTT CTTGGCACGA TTAACCCTGA TACCAATAAA ATCCCTAAGC ATTTGTTTCA GGGTTATTTG ATATCTATAG CGTATTTTAA AGCGCCGTGG ---ATGCCTG ACCGTACCGA
1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440

A1/12c A12c/13
GGCTAACCCT AATGAGCTTA ATCAAGATGA TGCTCGTTAT GGTTTCCGTT GCTGCCATCT CAAAAACATT TGGACTGCTC CGCTTCCTCC TGAGACTGAG CTTTCTCGCC AAATGACGAC
1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560

A13/2 M3/4
TTCTACCACA TCTATTGACA TTATGGGTCT GCAAGCTGCT TATGCTAATT TGCATACTGA CCAAGAACGT GATTACTTCA TGCAGCGTTA CCATGA-GTT ATTTCTTCAT TTGGAGGTAA
1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680

H5/9a Z4/1
AACCTCATAT GACGCTGACA ACCGTCCTTT ACTTGTCATG CGCTCTAATC TCTGGGCATC TGGCTATGAT GTTGATGGAA CTGACCAAAC GTCGTTAGGC CAGTTTTCTG GTCGTGTTCA
1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800

H9a/8a F2/11
ACAGACCTAT AAACATTCTG TGCCGCGTTT CTTTGTTCCT GAGCATGGCA CTATGTTTAC TCTTGCGCTG GTTCGTTTTC CGCCTACTGC GACTAAAGAG ATTCAGTACC TTAACGCTAA
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920

Q3c/6 F11/7
AGGTGCTTTG ACTTATACCG ATATTGCTGG CGACCCTGTT TTGTATGGCA ACTTGCCGCC GCGTGAAATT TCTATGAAGG ATGTTTTCCG TTCTGGTGAT TCGTCTAAGA AGTTTAAGAT
1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040

H8a/6 Q6/5 Q5/3b
TGCTGAGGGT CAGTGGTATC GTTATGCGCC TTCGTATGTT TCTCCTGCTT ATCACCTTCT TGAAGGCTTC CCATTCATTC AGGAACCGCC TTCTGGTGAT TTGCAAGAAC GCGTACTTAT
2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160

F7/5b
TCGCAACCAT GATTATGACC AGTGTTTCAG TCGTTCAGTT GTTGCAGTGG ATAGTCTTAC CTCATGTGAC GTTTATCGCA ATCTGCCGAC CACTCGCGAT TCAATCATGA CTTCGTGATA
2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280
End F ↑

Fig. 1 For full description see p. 690.

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                                                       R1/9       H6/3
AAAGATTGAG TGTGAGGTTA TAACCGAAGC GGTAAAAATT TTAATTTTTG CCGCTGAGGG GTTGACCAAG CGAAGCGCGG TAGGTTTTCT GCTTAGGAGT TTAATCATGT TTCAGACTTT
      2290       2300       2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
                                                                                                            G start ↑

                                       A2/16                        A16/15a H4/10                        A15a/3     R9/10
TATTTCTCGC CACAATTCAA ACTTTTTTTC TGATAAGCTG GTTCTCACTT CTGTTACTCC AGCTTCTTCG GCACCTGTTT TACAGACACC TAAAGCTACA TCGTCAACGT TATATTTTGA
      2410       2420       2430       2440       2450       2460       2470       2480       2490       2500       2510       2520

                                  M10/9                  R10/2
TAGTTTGACG GTTAATGCTG GTAATGGTGG TTTTCTTCAT TGCATTCAGA TGGATACATC TGTCAACGCC GCTAATCAGG TTGTTTCAGT TGGTGCTGAT ATTGCTTTTG ATGCCGACCC
      2530       2540       2550       2560       2570       2580       2590       2600       2610       2620       2630       2640

                               F5b/8  M9/2
TAAATTTTTT GCCTGTTTGG TTCGCTTTGA GTCTTCTTCG GTTCCGACTA CCCTCCCGAC TGCCTATGAT GTTTATCCTT TGGATGGTCG CCATGATGGT GGTTATTATA CCGTCAAGGA
      2650       2660       2670       2680       2690       2700       2710       2720       2730       2740       2750       2760

                                  Y2/5                                                                              F8/4
CTGTGTGACT ATTGACGTCC TTCCCCGTAC GCCCGGCAAT AACGTCTACG TTGGTTTCAT GGTTTGGTCT AACTTTACCG CTACTAAATG CCGCGGATTG GTTTCGCTGA ATCAGGTTAT
      2770       2780       2790       2800       2810       2820       2830       2840       2850       2860       2870       2880

                                    Q3b/4                                                  H3/2
TAAAGAGATT ATTTGTCTCC AGCCACTTAA GTGAGGTGAT TTATGTTTGG TGCTATTGCT GGCGGTATTG CTTCTGCTCT TGCTGGTGGC GCCATGTCTA AATTGTTTGG AGGCGGTCAA
      2890       2900       2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
                            End G ↑  H start ↑

            Y5/4       Q4/7                                  Q7/2
AAAGCCGCCT CCGGTGGCAT TCAAGGTGAT GTGCTTGCTA CCGATAACAA TACTGTAGGC ATGGGTGATG CTGGTATTAA ATCTGCCATT CAAGGCTCTA ATGTTCCTAA CCCTGATGAG
      3010       3020       3030       3040       3050       3060       3070       3080       3090       3100       3110       3120

Z1/2                                A3/9
GCCGCCCCTA GTTTTGTTTC GTGTGCTATT GCTAAAGCTG GTAAAGGACT TCTTGAAGGT ACGTTGCAGG CTGGCACTTC TGCCGTTTCT GATAAGTTGC TTGATTTGGT TGGACTTGGT
      3130       3140       3150       3160       3170       3180       3190       3200       3210       3220       3230       3240

                                                                  A9/12d                                                R2/6a
GGCAAGTCTG CCGCTGATAA AGGAAAGGAT ACTCGTGATT ATCTTGCTGC TGCATTTCCT GAGCTTAATG CTTGGGAGCG TGCTGGTGCT GATGCTTCCT CTGCTGGTAT GGTTGACGCC
      3250       3260       3270       3280       3290       3300       3310       3320       3330       3340       3350       3360

Y4/1   F4/14a    A12d/7c                                                    F14a/12
GGATTTGAGA ATCAAAAAGA GCTTACTAAA ATGCAACTGG ACAATCAGAA AGAGATTGCC GAGATGCAAA ATGAGACTCA AAAAGAGATT GCTGGCATTC AGTCGGCGAC TTCACGCCAG
      3370       3380       3390       3400       3410       3420       3430       3440       3450       3460       3470       3480

                                                             F12/10
AATACGAAAG ACCAGGTATA TGCACAAAAT GAGATGCTTG CTTATTC-AC AGAAGGAGTC TACTGCTGCG TTGCGTCTAT TATGGAAAAC ACCAATCTTT CCAAGCAACA GCAGGTTTCC
      3490       3500       3510       3520       3530       3540       3550       3560       3570       3580       3590       3600

     H2/9b               A7c/8                                               F10/15                R6a/4
GAGATTATGC GCCAAATGCT TACTCAAGCT CAAACGGCTG GTCAGTATTT TACCAATGAC CAAATCAAAG AAATGACTCG CAAGGTTAGT GCTGAGGTTG ACTTAGTTCA TCAGCAAACG
      3610       3620       3630       3640       3650       3660       3670       3680       3690       3700       3710       3720

 F15/5c       M2/5         H9b/1                                                                                    A8/14
CAGAATCAGC GGTATGGCTC TTCTCATATT GGCGCTACTG CAAAGGATAT TTCTAATGTC GTCACTGATG CTGCTTCTGG TGTGGTTGAT ATTTTTCATG GTATTGATAA AGCTGTTGCC
      3730       3740       3750       3760       3770       3780       3790       3800       3810       3820       3830       3840

                                 A14/7b
GATACTTGGA ACAATTTCTG GAAAGACGGT AAAGCTGATG GTATTGGCTC TAATTTGTCT AGGAAATAAC CGTCAGGATT GACACCCTCC CAATTGTATG TTTTCATGCC TCCAAATCTT
      3850       3860       3870       3880       3890       3900       3910       3920       3930       3940       3950       3960
                                                             End H ↑                                         mRNA start ↑

                                                                              T1/6
GGAGGCTTTT TTATGGTTCG TTCTTATTAC CCTTCTGAAT GTCACGCTGA TTATTTTGAC TTTGAGCGTA TCGAGGCTCT TAAACCTGCT ATTGAGGCTT GTGGCATTTC TACTCTTTCT
      3970       3980       3990       4000       4010       4020       4030       4040       4050       4060       4070       4080
  A start ↑

                                                 A7b/7a    M5/8  F5c/3                        T6/2     Q2/3a          R4/3  Z2/6b
CAATCCCCAA TGCTTGGCTT CCATAAGCAG ATGGATAACC GCATCAAGCT CTTGGAAGAG ATTCTGTCTT TTCGTATGCA GGGCGTTGAG TTCGATAATG GTGATATGTA TGTTGACGGC
      4090       4100       4110       4120       4130       4140       4150       4160       4170       4180       4190       4200

CATAAGGCTG CTTCTGACGT TCGTGATGAG TTTGTATCTG TTACTGAGAA GTTAATGGAT GAATTGGCAC AATGCTACAA TGTGCTCCCC CAACTTGATA TTAATAACAC TATAGACCAC
      4210       4220       4230       4240       4250       4260       4270       4280       4290       4300       4310       4320

                                            M8/6                   A7a/4
CGCCCCGAAG GGGACGAAAA ATGGTTTTTA GAGAACGAGA AGACGGTTAC GCAGTTTTGC AAGCTGGCTG CTGAACGCCC TCTTAAGGAT ATTCGCGATG AGTATAATTA CCCCAAAAAG
      4330       4340       4350       4360       4370       4380       4390       4400       4410       4420       4430       4440

                                    Z6b/6a
AAAGGTATTA AGGATGAGTG TTCAAGATTG CTGGAGGCCT CCACTAAGAT ATCGCGTAGA GGCTTTGCTA TTCAGCGTTT GATGAATGCA ATGCGACAGG CTCATGCTGA TGGTTGGTTT
      4450       4460       4470       4480       4490       4500       4510       4520       4530       4540       4550       4560

ATCGTTTTTG ACACTCTCAC GTTGGCTGAC GACCGATTAG AGGCGTTTTA TGATAATCCC AATGCTTTGC GTGACTATTT TCGTGATATT GGTCGTATGG TTCTTGCTGC CGAGGGTCGC
      4570       4580       4590       4600       4610       4620       4630       4640       4650       4660       4670       4680
```

```
         F3/5a                                                 A4/11   Z6a/9  M6/1
AAGGCTAATG ATTCACACGG CGACTGCTAT CAGTATTTTT GTGTGCCTGA GTATGGTACA GCTAATGGCC GTCTTCATTT CCATGCGGTG CACTTTATGC GGACACTTCC TACAGGTAGC
      4690       4700       4710       4720       4730       4740       4750       4760       4770       4780       4790       4800

 R3/8                                           A11/10               Z9/10
GTTGACCCTA ATTTTGGTCG TCGGGTACGC AATCGCCGCC AGTTAAATAG CTTGCAAAAT ACGTGGCCTT ATGGTTACAG TATGCCCATC GCAGTTCGCT ACACGCAGGA CGCTTTTTCA
      4810       4820       4830       4840       4850       4860       4870       4880       4890       4900       4910       4920

                Z10/3                Q3a/1       A10/12b                                           R8/5
CGTTCTGGTT GGTTGTGGCC TGTTGATGCT AAAGGTGAGC CGCTTAAAGC TACCAGTTAT ATGGCTGTTG GTTTCTATGT GGCTAAATAC GTTAACAAAA AGTCAGATAT GGACCTTGCT
      4930       4940       4950       4960       4970       4980       4990       5000       5010       5020       5030       5040

            A12b/15b                            A15b/17              A17/12a   F5a/6
GCTAAAGGTC TAGGAGCTAA AGAATGGAAC AACTCACTAA AAACCAAGCT GTCGCTACTT CCCAAGAAGC TGTTCAGAAT CAGAATGAGC CGCAACTTCG GGATGAAAAT GCTCACAATG
      5050       5060       5070       5080       5090       5100       5110       5120       5130       5140       5150       5160
                         ↑
                 B start |

                                       A12a/5
ACAAATCTGT CCACGGAGTG CTTAATCCAA CTTACCAAGC TGGGTTACGA CGCGACGCCG TTCAACCAGA TATTGAAGCA GAACGCAAAA AGAGAGATGA GATTGAGGCT GGGAAAAGTT
      5170       5180       5190       5200       5210       5220       5230       5240       5250       5260       5270       5280

                       H1/15                               H15/8b  T2/7                            P1/1
ACTGTAGCCG ACGTTTTGGC GGCGCAACCT GTGACGACAA ATCTGCTCAA ATTTATGCGC GCTTCGATAA AAATGATTGG CGTATCCAAC CTGCA
      5290       5300       5310       5320       5330       5340       5350       5360       5370  5375
```

Fig. 1 A provisional nucleotide sequence for the DNA of bacteriophage ΦX174 *am*3 *cs*70. Solid underlining indicates sequences that are fully confirmed; sequences with no underlining probably do not contain more than one mistake per 50 residues. Broken underlining indicates more uncertain sequences. Restriction enzyme recognition sites are indicated (for key to single letter enzyme code see legend to Fig. 2), as are mRNA starts and protein initiation and termination sites. Nucleotides 4,127 to 4,201 have been independently sequenced by van Mansfield *et al.*[illegible]. The *am*3 codon is at position 587.

be eliminated by more laborious experiments and, although much of the sequence has been so confirmed, it would probably be a long time before the complete sequence could be established. We are not certain that there is any scientific justification for establishing every detail and, as it is felt that the results may be useful to other workers, it has been decided to publish the sequence in its present form.

As template we have used both the viral (plus) and complementary (minus) strands of ΦX. Usually it is possible to determine a sequence with a single primer starting at about 15–100 nucleotides from the appropriate restriction enzyme site. In a particularly good experiment the sequence can be read out to 150–200 nucleotides but the results may become less reliable. Most sequences have been derived by priming on both strands; this allows more confidence than when only one strand could be used.

A useful method for confirming runs of the same nucleotide is depurination of ^{32}P-labelled small restriction enzyme fragments or of products of the DNA polymerase priming experiments (ref. 31 and N.L.B. and M.S., in preparation). The most satisfactory way of confirming the DNA sequences is through amino acid sequence data. As the methods used are entirely unrelated, the results of the two approaches complement each other very well and therefore complete sequences can usually be deduced from incomplete data obtained by each method. The complete sequence of genes *G* (ref. 32), *D* (ref. 33), *J* (ref. 33 and Freymeyer, unpublished) and most of *F* have been obtained in this way.

Many of the sequences in Fig. 1 have been amply confirmed and are regarded as established: these are indicated in the figure by underlining. Some sequences are considered to be reasonably accurate and probably contain no more than one mistake in every 50 nucleotides. Sequences that are particularly uncertain—either because of lack of data or conflicting results—are also indicated in Fig. 1.

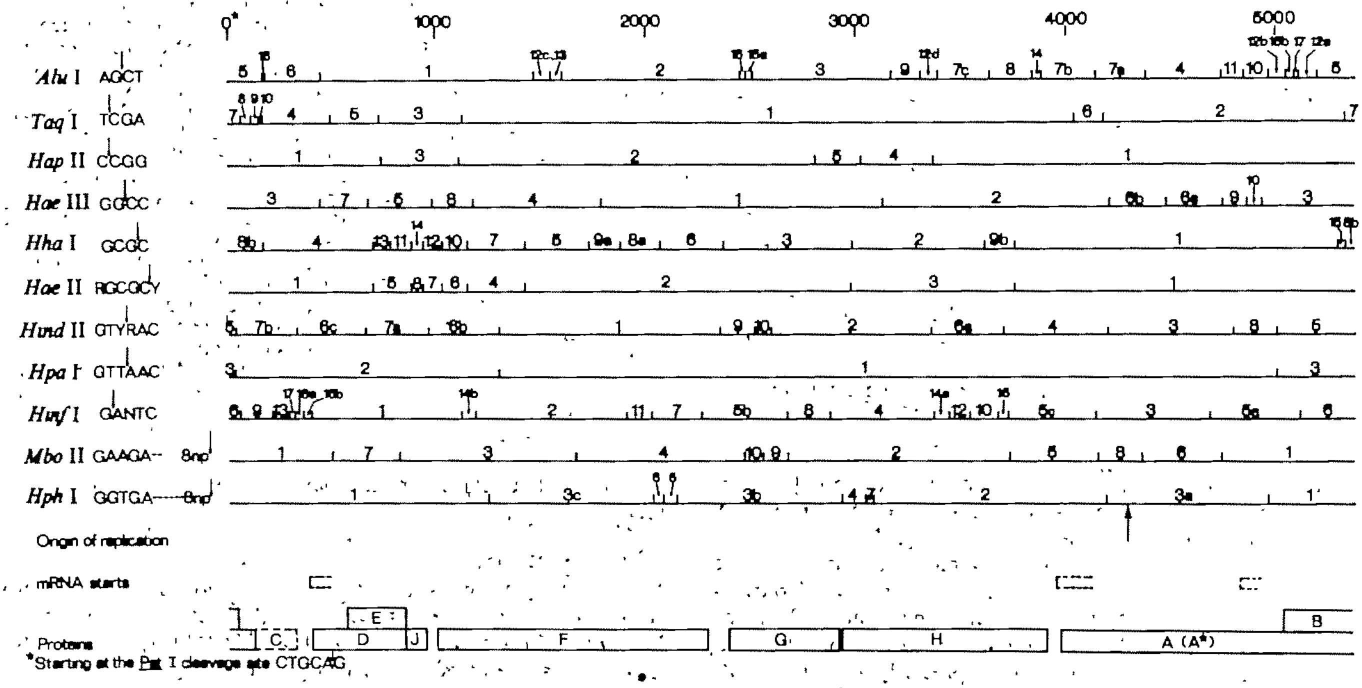
In considering the sequence of ΦX174 as a functional unit it is convenient to begin in the region between the *H* and *A* genes and to continue around the DNA in the direction of transcription and translation.

A promoter and terminator

Sinsheimer *et al.*[34,35] and Axelrod[36] have determined the sequences of the 5′ end of three ΦX *in vitro* mRNA species and have located them on the restriction map. These sequences have been identified on the DNA sequence and one of them (AAATCTTGG) is found only at position 3,954 at which an *in vivo* unstable mRNA start has been located[37]. The sequence to the left of this has some characteristics of typical *E. coli* promoters[38] in that five out of the 'ideal' TATPuATPu residues are present. Nearby, to the right of this mRNA initiation, however, is the sequence TTTTTTA which is similar to sequences found at the 3′ ends of a number of mRNAs (see ref. 39) and seems a likely signal for mRNA termination. The presence of a rho-independent termination site in this approximate position has been suggested[36,37], but the relative positions of the initiating and putative termination signals is rather surprising since the terminator for one mRNA would be expected to precede the initiator for the next. One possibility is that the T_4A might be acting as an 'attenuator' involved in the control of mRNA production in a similar manner to that suggested for the tryptophan operon by Bertrand *et al.*[40]. If indeed it were acting as a transcription terminator one would expect a small RNA of 20 nucleotides to be produced, but no such product has yet been detected. Recent work, however, (Rosenberg, unpublished and ref. 41) indicates that termination may require the presence of a base-paired loop structure before the termination site. From the DNA sequence such a loop is probably present before the T_4A sequence, but in mRNA starting from the initiation site at position 3,954 this loop is not formed (Fig. 3). Therefore mRNA that had started at an earlier promoter and extended through the *H* gene would be expected to terminate here, whereas mRNA newly initiated at position 3,954 would not. This could be a way in which the phage has economised on the use of DNA—by having the ends of the two mRNAs overlapping.

The A protein

Where the amino acid sequence is available there is no problem in relating the DNA sequence to its coding properties, but it is



*Starting at the Pst I cleavage site CTGCAG

Fig. 2 Fragment maps of restriction enzymes used in the sequence analysis of ΦX174 *am*3 RFI DNA. Fragment maps of ΦX174 have been prepared for *Hin*dII (R), *Hae*III (Z) and *Hpa*I+II by Lee and Sinsheimer[35], *Hin*HI and *Hap*II (Y) by Hayashi and Hayashi[36], and for *Alu*I (A) by Vereijken *et al.*[37] and for *Pst*I (P) by Brown and Smith[38]. B.G.B., G.M.A., C.A.H. and D. Jaffe prepared the *Hin*fI (F) map, C.A.H. the *Hph*I (Q) map, and Jeppesen *et al.*[33] the *Hha*I (H), *Alu*I, *Hae*II and *Hap*II maps by using a rapid method depending on priming with DNA polymerase. A rapid two-dimensional hybridisation technique has been developed by C.A.H. (submitted for publication) and recently used for mapping *Mbo*II (M) (N.L.B., C.A.H., and M.S., submitted for publication) and *Taq*I (T)[39]. *Hha*I and *Hin*fI maps have also been prepared by Baas *et al.*[33].

more difficult to do so in the absence of such data, as is the case for the A protein. One way of identifying the reading phase of the DNA is from the distribution of nonsense codons. Over a sufficiently long sequence that is known to be coding for a single protein there is usually one phase that contains no nonsense codons, and this is identified as the reading phase. This requires completely accurate determination of the DNA sequences however: omission of a single nucleotide may give completely erroneous results. Another approach is possible in the case of ΦX. The results with the *F* and *G* genes[13,34,35] showed an unexpectedly high frequency of codons ending in T. Therefore in a coding region there is a tendency for every third nucleotide to be a T and it is then possible to define the reading phase. Figure 4 illustrates how this characteristic was used to help determine the reading phase for the A protein and to identify its initiation codon at position 3,973. In a similar way the distribution of Ts may be used to identify errors in the DNA sequence, provided that such errors occur only infrequently.

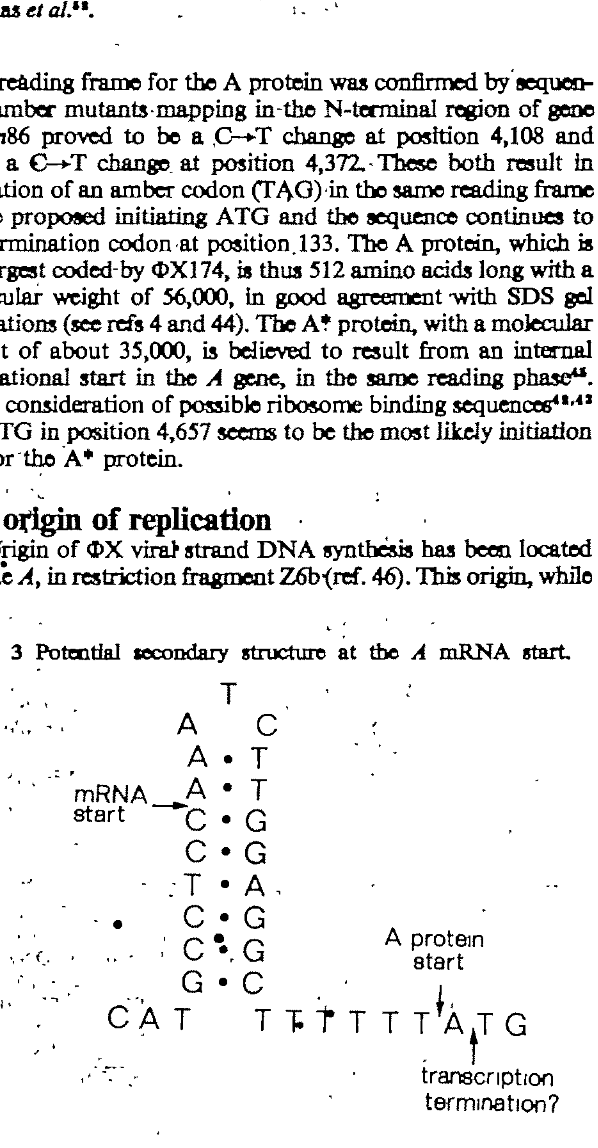
A different approach to identifying the initiation site and reading phase in a coding sequence is by looking for a characteristic 'initiation sequence'. Shine and Dalgarno have shown that a common feature of ribosome binding sites is a number of nucleotides (at least three) preceding the ATG that are capable of forming base pairs with a sequence at the 3′ end of 16S rRNA[42,43]. All of the known initiation sites in ΦX174 that have been identified by direct amino acid sequencing (for the F, G, H, J and D proteins) satisfy this criterion (see Table 2) and the fact that the sequence preceding the ATG in position 3,973 also has this characteristic supports its identification as the initiation site for the A protein.

If, as has been suggested[37], some mRNA from the previous promoter does extend beyond the hairpin structure, initiation of A protein synthesis may be controlled by the inclusion of the region complementary to the 16S rRNA in the hairpin loop. This could explain the presence of two types of mRNA covering the *A* cistron, as suggested by Hayashi *et al.*[37]—one unstable and active and the other stable but inactive. The former would be intiated at the A promoter, the latter at an earlier promoter and result from 'read-through' at the terminator. The postulated reading frame for the A protein was confirmed by sequencing amber mutants mapping in the N-terminal region of gene *A*. *am*86 proved to be a C→T change at position 4,108 and *am*33 a C→T change at position 4,372. These both result in formation of an amber codon (TAG) in the same reading frame as the proposed initiating ATG and the sequence continues to the termination codon at position 133. The A protein, which is the largest coded by ΦX174, is thus 512 amino acids long with a molecular weight of 56,000, in good agreement with SDS gel estimations (see refs 4 and 44). The A* protein, with a molecular weight of about 35,000, is believed to result from an internal translational start in the *A* gene, in the same reading phase[45]. From consideration of possible ribosome binding sequences[42,43] the ATG in position 4,657 seems to be the most likely initiation site for the A* protein.

The origin of replication

The origin of ΦX viral strand DNA synthesis has been located in gene *A*, in restriction fragment Z6b (ref. 46). This origin, while

Fig. 3 Potential secondary structure at the *A* mRNA start.



```
                                                      Ts in codon
                                                       position
                                                      (1) (2) (3)
3,910
  CCG.TCA.GGA.TTG.ACA.CCC.TCC.CAA.TTG.TAT.             5   2   1
                                                       3   4   3*
3,940
  GTT.TTC.ATG.CCT.CCA.AAT.CTT.GGA.GGC.TTT.             2   5   5
                                                       3   5   7*
3,970
  TTT.ATG.GTT.CGT.TCT.TAT.TAC.CCT.TCT.GAA.             5   3   7
                                                       5   0   7*
4,000
  TGT.CAC.GCT.GAT.TAT.TTT.GAC.TTT.GAG.CGT.             4   2   7
```

*These figures refer to the last five codons of the previous line and the first five of the next line.

Fig. 4 Identification of the initiation codon for the A protein. Sequences of 30 nucleotides in the region in which the initiation was expected were written down and arbitrarily marked off in triplets. The number of T residues in the first position in each triplet was then counted and listed, and similarly the number of Ts in the second and third positions. The marked preference for Ts in position 3 in the last two lines, as compared with the first two lines, suggests that they are coding for protein and that the triplets are correctly marked off. The most likely initiation codon for the A protein is the ATG in position 3,973.

coding for part of the A protein, probably corresponds to the position of the plus strand nick made by the same protein[44]. Gaps in this region that are found in replicating double-stranded (RF) DNAs are probably related to the position of the nick. Eisenberg *et al.*[47] have investigated such gaps by depurination analysis and identified, in particular, the product C_4T. The sequence CTC_4 is found in position 4,285 (Fig. 1) and the location of the origin in this region agrees precisely with the results of Baas *et al.*[44]. It is not possible at present to identify the actual position of the origin nick. The region shows no apparent secondary structure or symmetrical sequences, although there is an AT-rich region (4,298–4,307) between two GC-rich regions which might be of significance. Such a region is found near the origin of replication of SV40 DNA (ref. 48).

B promoter

The second of the mRNA 5′ sequences (AUCGC)[34] has been mapped in restriction fragment R8 (Fig. 2), which starts about 300 nucleotides on from the proposed A* initiation. The sequence ATCGC is found at positions 4,832 and 4,888 in Fig. 1. The only way we can choose between them at the moment is that the second is preceded by the sequence TACAGTA (position 4,877), which is more akin to sequences found in known promoters[38] than are sequences preceding the other possible mRNA start. Irrespective of which of these sequences is used, the mRNA has a long 'leader' sequence (232 or 176 nucleotides) before the next proposed initiation codon (gene *B*).

Table 1 ΦX174 coding capacity

Gene	Protein molecular weight from SDS gels*	Number of nucleotides (Fig. 1)	Protein molecular weight from sequence information
A	55,000–67,000	1,536	56,000
(*A**)	35,000		
B	19,000–25,000	(360)†	13,845‡
C	7,000		
D	14,500	456	16,811‡
E	10,000–17,500	(273)†	9,940
J	5,000	114	4,097‡
F	48,000	1,275	46,400
G	19,000	525	19,053‡
H	37,000	984	35,800
Non-coding and *C*		485	
Total		5,375	

*See ref. 4

†Values in parenthesis are overlapping sequences and therefore not included in the addition to obtain the total length of DNA.

‡These values are calculated from the amino acid sequence (in the case of B deduced from the nucleotide sequence). The others are derived using the formula

$$\text{Protein molecular weight} = \frac{\text{No. of nucleotides}}{3\times 0.00915}$$

The B protein

From a study of the ribonuclease T_1 digestion products of the ribosome binding sites of ΦX mRNAs[49], it was possible to identify an initiating ATG in position 5,064. From the genetic map[2,3], this would be expected to be gene *B* but, as discussed above, the A protein coding sequence extends right through this region, past the *Pst* site at residue 1 in Fig. 1, and terminates at residue 133. The initiating codon contained in the ribosome-protected sequence is, however, out of phase with the A protein reading frame. The proposed B protein coding sequence is one nucleotide to the left of the A protein phase, and continues until a termination codon occurs at position 49. Therefore the B protein coding sequence is totally contained within the *A* gene. These reading frames have been confirmed by sequencing mutants in genes *A* and *B* (*am*16, N.L.B. and M.S., in preparation; *am*18, *am*35, *ts*116 (ref. 50). Since the B protein has not been purified no protein sequence data is available. The complete amino acid sequence can be predicted from the DNA sequence however. The protein is 120 amino acids long with a molecular weight of 13,845 (including the N-terminal Met). The molecular weight estimates of the B protein obtained by SDS–gel electrophoresis are mostly greater than this (see review, ref. 4), but the electrophoretic mobility varied with gel concentration and cross linker. Such anomalous behaviour suggests that there may be, for instance, carbohydrate attached to the B protein.

The C protein

The next known gene product, protein C, maps between genes *B* and *D*. Examination of the DNA sequence in this region indicates that the most probable initiating ATG overlaps the termination codon, TGA, of gene A in the sequence ATGA at position 134. A possible termination codon for gene *C* could then be at position 391, although the sequence and phasing is not yet confirmed through this region. There is another possible protein initiation codon (position 51, overlapping the B protein termination codon) which would result in a slightly shorter gene product terminating at nucleotide 219. For the C protein, however, we favour the 'A terminator' start, since only this reading frame contains a CAA sequence, which by a C→T alteration could give the ochre 6 mutant. Ochre 6 is a gene *C* mutant produced by the decay of ^{3}H-cytosine[51] and has been mapped in fragments A6 and F9 (ref. 52); that is, between nucleotides 170 and 205 (Fig. 1).

Sequence following the *D* promoter

The mRNA 5′ sequence which maps before the *D* gene (GAUGC)[34] is found at position 358 in Fig. 1. The sequence preceding the messenger start has only four of the TATPuATPu nucleotides[38]. Thirty-two nucleotides after the mRNA initiation is the ATG (position 390) that initiates D protein synthesis. The amino acid sequence of the D protein has been determined

Table 2 Initiation sequences of ΦX174 coded proteins

D	C-C-A-C-T-A-A-T-A-G-G-T-A-A-G-A-A-A-T-C-A-T-G-A-G-T-C-A-A-G-T-T-A-C-T	Ser Gln Val Thr
E	C-T-G-C-G-T-T-G-A-G-G-C-T-T-G-C-G-T-T-T-A-T-G-G-T-A-C-G-C-T-G-G-A-C-T	
J	C-G-T-G-C-G-G-A-A-G-G-A-G-T-G-A-T-G-T-A-A-T-G-T-C-T-A-A-A-G-G-T-A-A-A	Ser Lys Gly Lys
F	C-C-C-T-T-A-C-T-T-G-A-G-G-A-T-A-A-A-T-T-A-T-G-T-C-T-A-A-T-A-T-T-C-A-A	Ser Asn Ile Gln
G	T-T-C-T-G-C-T-T-A-G-G-A-G-T-T-T-A-A-T-C-A-T-G-T-T-T-C-A-G-A-C-T-T-T-T	Met Phe Gln Thr Phe
H	C-C-A-C-T-T-A-A-G-T-G-A-G-G-T-G-A-T-T-T-A-T-G-T-T-T-G-G-T-G-C-T-A-T-T	Met Phe Gly Ala Ile
A	C-A-A-A-T-C-T-T-G-G-A-G-G-C-T-T-T-T-T-T-A-T-G-G-T-T-C-G-T-T-C-T-T-A-T	
B	A-A-A-G-G-T-C-T-A-G-G-A-G-C-T-A-A-A-G-A-A-T-G-G-A-A-C-A-A-C-T-C-A-C-T	
16S RNA 3′ end	HO A-U-U-C-C-U-C-C-A-C-U-A-G	

Where the protein start has been independently confirmed by protein sequencing data the amino acid sequences are indicated. The other initiation regions were identified as described in the text. Sequences complementary to the 3′ end of 16S rRNA (refs 42, 43) are boxed; broken lines indicate further complementarity if some nucleotides are looped out or not matched. Ribosome binding to mRNA has been demonstrated in these regions for genes *J*, *F*, *G* and *B* (ref. 49).

almost completely, and nucleotide and amino acid sequences can be correlated to the termination codon at position 846 (ref. 33). The D protein, which is involved in capsid assembly, is 151 amino acids in length, with a molecular weight of 16,811. The *D* termination codon overlaps the initiation codon for gene *J* in the sequence TAATG. A similar structure has also been found by Platt and Yanofsky[53] in the tryptophan operon. The DNA sequence following this initiation codon matches the amino acid sequence of the small basic protein (37 amino acids) of the virion determined by D. Freymeyer, P. R. Shank, T. Vanaman, C.A.H. and M. H. Edgell (personal communication). Benbow *et al.*[8,9] suggested that the mutation *am*6 was located in a gene *J*, coding for the small protein of the virion, and mapping immediately before gene *F*. Although marker rescue experiments indicate that *am*6 is not in this region[54], the DNA sequence shows that there is a gene coding for the virion protein and we

Table 3 Promoter sequences in ΦX174

A promoter	A-G-G-A-T-T-G-A-C-A-C-C-C-T-C-C-C-A-A-T-T-G-T-A-T-G-T-T-T-T-C-A-T-G-C-C-T-C-C-A-A-A-T-C-T - - -	3954	18 nucleotides to A′ protein start
D promoter	R7b/R6o G-T-T-G-A-C-A-T-T-T-T-A-A-A-A-G-A-G-C-G-T-G-G-A-T-T-A-C-T-A-T-C-T-G-A-G-T-C-C-G-A-T-G-C-T	358	32 nucleotides to D protein start
B promoter?	R3/R8 C-A-G-G-T-A-G-C-G-T-T-G-A-C-C-C-T-A-A-T-T-T-T-G-G-T-C-G-T-C-G-G-T-A-C-G-C-A-A-T-C-G-C-C	4832	232 nucleotides to B protein start
B promoter?	A-G-C-T-T-G-C-A-A-A-A-T-A-C-G-T-G-G-C-C-T-T-A-T-G-G-T-T-A-C-A-G-T-A-T-G-C-C-C-A-T-C-G-C-A	4888	176 nucleotides to B′ protein start

mRNA initiation sequences[44–46] are underlined. Boxed regions indicate sequences that may correspond to the TATPuATPu sequence found in other promoters[48], taking into account the distance from the mRNA starts.

have defined this as gene *J* (ref. 33). Since the *J* initiation codon overlaps the *D* termination codon we had to look elsewhere for gene *E*, which genetic mapping[2,3] had placed between them. Amber mutants in gene *E* (*am*3, *am*27, *am*34 and *am*N11) were located by the marker rescue technique and sequenced. All were found to be within the *D* coding sequence, with the mutant amber codons one nucleotide to the right of the *D* reading frame[33]. Thus the *E* coding sequence is completely contained within the *D* coding region but in a different reading frame. The proposed initiation and termination codons for the E protein are at nucleotides 568 and 840, respectively[33], giving a protein 91 amino acids in length with a molecular weight of about 9,900 (including the N-terminal methionine).

The F protein

Following the *J* gene is an intercistronic region of 39 nucleotides before initiation of the F protein. There is no known function of this apparently untranslated sequence, although the presence of a hairpin structure (positions 969–984) suggests that it could be the site of the *in vivo* messenger termination signal [37] mapped in this region. The F protein is initiated by the ATG at position 1,001. This is the capsid component of the virion, and almost all the amino acid sequence is known[33,34]. There are regions in this gene where the DNA sequence is not completely established, but the protein is about 424 amino acids in length, giving a molecular weight of ≃46,300.

The G protein region

The termination signal for the F protein (position 2,276) is followed by an unusually long untranslated sequence of 111 nucleotides until the G protein initiation codon[31]. This region contains a looped structure which was postulated to have some functional role, as yet unknown, in the single-stranded DNA or the mRNA.

Initiation of the G protein at position 2,387 is followed by a sequence of 425 nucleotides until termination at position 2,912, giving a spike protein of molecular weight 19,053. The nucleotide and amino acid sequences of this gene and product are known[34,35].

The H protein

The initiation codon for the H protein (position 2,923) was identified first on the basis of the distribution of T nucleotides between the three reading phases, and later confirmed by amino acid sequence analysis. Amino acid sequence data on the H protein is minimal but the five peptide sequences known do correspond to the amino acid sequence, deduced from the DNA sequence by using the high frequency of third position T to help in assigning a reading frame to any given region. The DNA sequence is not entirely confirmed but it is possible to write a reasonably accurate amino acid sequence for the H protein. The protein terminates at nucleotide 3,907, in agreement with carboxypeptidase results, giving a spike protein of molecular weight ≃ 35,600 (326 amino acids). The amino acid sequence at the N terminus seems to be particularly rich in hydrophobic residues, which is consistent with its suggested function as the 'pilot' protein that reacts with the bacterial membrane[45,46]. After H protein termination there are 66 nucleotides before initiation of the A protein at position 3,973.

Coding capacity of the ΦX174 genome

The most striking feature of the ΦX DNA sequence is the way in which the various functions of the genome are compressed within the 5,375 nucleotides. Since the identification of ΦX gene products[3,4] it has been clear that proteins of the accepted molecular weights could not be separately coded on the available length of DNA. However, with the presence of two pairs of overlapping genes (*B* within *A* (ref. 50), *E* within *D* (ref. 33)) the genome has more coding capacity than had been originally supposed on the assumption that each gene was physically separate. Table 1 summarises the molecular weights of the known ΦX-coded proteins. There are other potential initiation sites for polypeptide synthesis (for example, in genes *A*, *F*, *G* and *H*) and further genetic work may clarify whether there are in fact other ΦX genes as yet unidentified.

Initiation of protein synthesis

Table 2 lists the protein initiation sequences for genes *A*, *B*, *D*, *E*, *J*, *F*, *G* and *H*. It can be noted that there are no extra precursor sequences in proteins D, J, F, G or H at either the N or C terminus. There seems to be no relationship between the degree of complementarity to the 16S rRNA and the amount of protein synthesised, and we see no other features in the sequence that could explain different efficiencies of translation except where genes overlap.

Transcription of ΦX174

The sequences preceding known mRNA starts[54–56] are shown in Table 3. Other studies on promoter sequences[58] have suggested certain features that they may have in common. Although some of these features are present in the sequences preceding the ΦX transcription initiations others are not, and at present it is difficult to suggest what signal on the DNA determines a promoter site or the efficiency with which it initiates RNA synthesis. It is interesting to note that a polymerase binding site found by Chen *et al.*[57], but not associated with any *in vitro* or *in vivo* mRNA starts, mapped near the region where there is the sequence TATGATG characteristic of promoters[58] (positions 2,705–2,711).

Table 4 Codons used in ΦX174

Phe	TTT	39	Ser	TCT	35	Tyr	TAT	36	Cys	TGT	12
	TTC	26		TCC	9		TAC	15		TGC	10
Leu	TTA	19		TCA	16	Ter	TAA	3	Ter	TGA	5
	TTG	26		TCG	14		TAG	0	Trp	TGG	16
Leu	CTT	36	Pro	CCT	34	His	CAT	16	Arg	CGT	40
	CTC	15		CCC	6		CAC	7		CGC	29
	CTA	3		CCA	6	Gln	CAA	27		CGA	4
	CTG	24		CCG	21		CAG	34		CGG	8
Ile	ATT	45	Thr	ACT	40	Asn	AAT	37	Ser	AGT	9
	ATC	12		ACC	18		AAC	25		AGC	5
	ATA	2		ACA	13	Lys	AAA	47	Arg	AGA	6
Met	ATG	42		ACG	19		AAG	31		AGG	1
Val	GTT	53	Ala	GCT	64	Asp	GAT	44	Gly	GGT	38
	GTC	14		GCC	17		GAC	35		GGC	28
	GTA	10		GCA	12	Glu	GAA	27		GGA	13
	GTG	11		GCG	12		GAG	34		GGG	3

The totals are derived from sequences in Fig. 1 which are fully confirmed, that is, 377 codons in gene *A*, 120 in gene *B*, 152 in gene *D*, 91 in gene *E*, 38 in gene *J*, 344 in gene *F*, 175 in gene *G* and 49 in gene *H*. Out of a total of 1,346 codons 42.9% terminate in T. The percentages in the different genes are: *A*, 37.1 (non-overlapping region 47.1; overlapping region 15.8); *B*, 34.2; *D*, 42.1; *E*, 14.3; *J*, 47.4; *F*, 52.0; *G*, 54.3; *H*, 49.0. The initiating ATG is included in all cases.

The use of codons in ΦX174

Table 4 shows the codons used in regions where the nucleotide sequence is fully confirmed. It is clear that the pattern established by early observations on non-random use of codons[23,24] is continued now that more information is available. In particular, the preference for T at the third position of the codon is marked throughout the genome, as shown in Table 4. In regions of overlapping genes, one of the pair tends to continue the 'third T' trend (*D* and *B*), thus excluding the other (*E* and *A*). This may give some indication of the order in which overlapping genes evolved[23,24]. Another interesting feature is the very low occurrence of codons starting AG, particularly in non-overlapping regions. The base composition of the sequence of ΦX174 DNA shown in Fig. 1 is: *A*, 23,9%; *C*, 21.5%; *G*, 23.3% and *T*, 31.2%. This is in good agreement with previously determined values (see ref. 4).

We thank D. McCallum and R. Staden for carrying out the computer data storage and analysis of the sequence.

Note added in proof: J. E. Sims and D. Dressler (personal communication) have independently determined the sequence in positions 263–375 and 4,801–4,940. Their results agree with those given in Fig. 1. They have also identified the 'B' mRNA start as being at position 4,888.

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DNA sequence of a region of the ΦX174 genome coding for a ribosome binding site

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The DNA region corresponding to a newly identified ribosome binding site in ΦX174 DNA is sequenced and mapped in relation to the physical map of ΦX174. Assignments of the binding site to a specific gene are discussed, and the possibility of a second case of overlapping genes in ΦX174 is considered.

RECENT studies on the DNA of bacteriophage ΦX174 have defined sequences corresponding to the ribosome binding sites of genes *D*, *J*, *F* and *G* (refs 1–3 and B. G. Barrell, personal communication). A ribosome binding site that does not correspond to any of the above sequences has now been defined by studies on a ribosome-protected fragment of a transcript *in vitro* of ΦX174 RF DNA (ref. 4). Inspection of preliminary DNA sequence data obtained from ΦX174 (F. Sanger, *et al.*, unpublished) for potential ribosome binding sites, and comparison of the sequence data with T_1-oligonucleotides from the unassigned RNA fragment[4], allowed identification of the DNA sequence corresponding to this RNA fragment. We report here the complete DNA sequence of a region of ΦX174 *am*3 RF DNA that corresponds to this new ribosome binding site. Because the DNA sequence contains accurately mapped cleavage sites for restriction endonucleases, the ribosome binding site can be located precisely on the physical map of the ΦX genome[5], and therefore located on the genetic map[6–8].

Determination of the DNA sequence

The ribosome binding site lies in that portion of *Hin*dII fragment 5 that overlaps *Hin*fI fragment 5a (nucleotides 5,000–5,100 approx, Fig. 1). The DNA sequence of this region was determined using the 'plus–minus' technique of Sanger and Coulson[9] (Fig. 2). It was quantitatively confirmed by characterisation of the pyrimidine oligonucleotides obtained by primed synthesis *in vitro* of DNA labelled with α-^{32}P-dATP or α-^{32}P-dGTP using viral or complementary strand as template[10,11] (Fig. 3, Table 1).

The sequence between the *Hin*dII 8/5 cleavage site and the *Hin*fI 5a/6 cleavage site contains 111 nucleotides. It also contains the *Alu*I cleavage sites between fragments 12b, 15b, 17 and 12a (Fig. 1). The sequence determination allowed the

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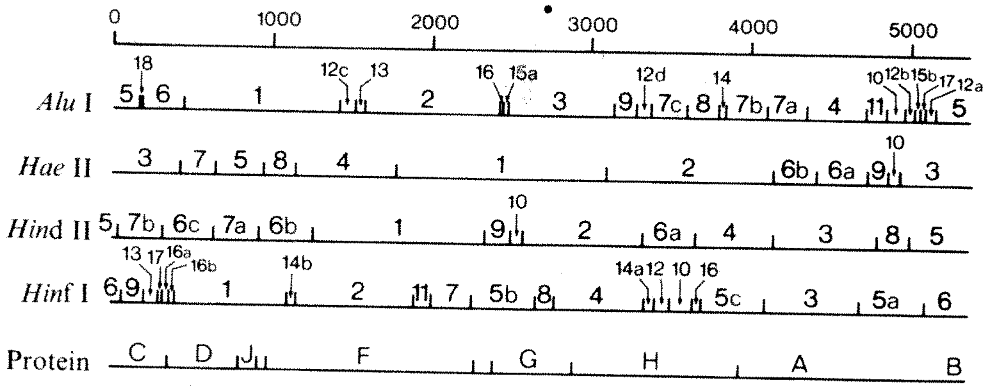

0
1000
2000
3000
4000
5000
Alu I
18
5 6 1 12c 13 2 16 15a 3 12d 9 7c 8 14 7b 7a 4 10 12b 15b 17 12a 11 5
Hae II
3 7 5 8 4 1 2 6b 6a 9 10 3
Hind II
5 7b 6c 7a 6b 1 9 10 2 6a 4 3 8 5
Hinf I
6 9 13 17 16a 16b 1 14b 2 11 7 5b 8 4 14a 12 10 16 5c 3 5a 6
Protein
C D J F G H A B

Fig. 1 Restriction endonuclease fragment map of ΦX174 *am*3 RFI DNA. The origin of this map is the *Pst*I cleavage site, the scale being the number of nucleotides from that site. The direction of translation is from left to right. The boundaries of genes *A*, *B* and *C* are not precisely defined. The sequence described in this paper is 111 nucleotides between positions, 5,000 and 5,110 approximately. The data on which the cleavage map is based come from the work of Lee and Sinsheimer[23] (*Hae*III and *Hin*dII), Vereijken *et al.*[12] (*Alu*I) and Jeppesen *et al.*[5] (*Hin*dII, *Alu*I and *Hin*fI) except that the correct ordering of *Alu*I fragments A12b, A15b, A17 and A12a results from this sequence determination.

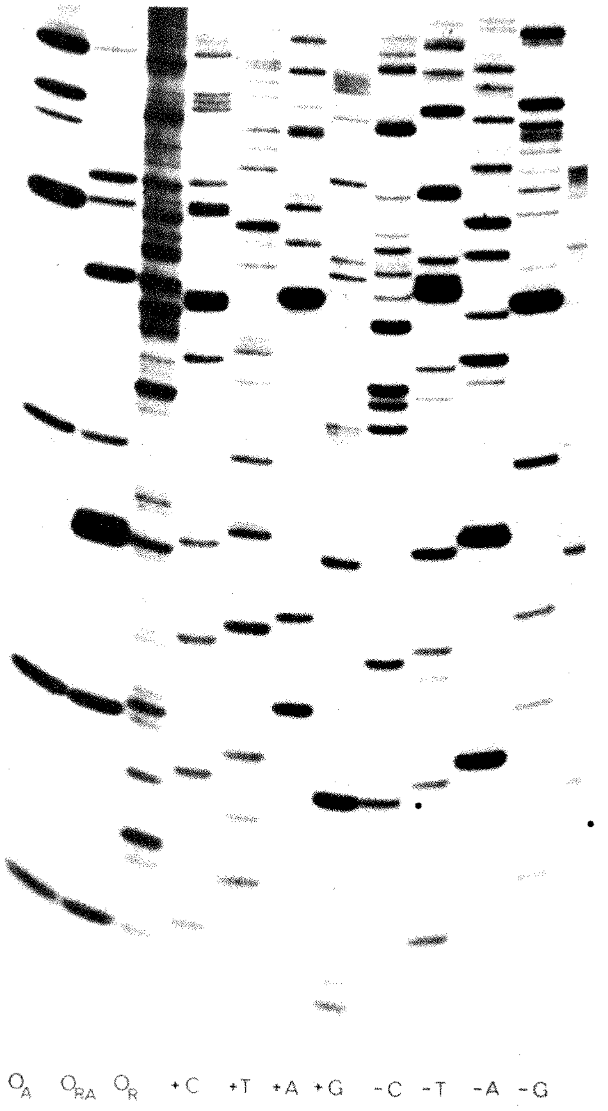


Fig. 2 The 'plus and minus' pattern produced by priming with *Hin*dII fragment R8 on complementary DNA template. This defines the sequence from nucleotide 19 to 88 (Fig. 4). The method used to locate the *Alu*I cleavage sites is that previously described for *Pst*I (ref. 24), except in that conditions were selected such that partial cleavage was obtained with *Alu*I. This allows location of all the *Alu*I sites in this region. The *Alu*I cleavage sites are defined by the two channels to the left of the plus and minus pattern, as bands which appear in channel O_{RA} but not in channel O_A.

correct ordering of *Alu*I fragments 12b, 15b, 17 and 12a for the first time[5,12]. The *Alu*I cleavage sites were mapped in relation to the plus–minus sequence determination as described in Fig. 2.

The sequence of the duplex DNA together with details of the determination is shown in Fig. 4.

Possible assignment of the ribosome binding site

As ΦX174 mRNAs are in the same sense as the viral strand[13], and the sequence corresponding to the RNA fragment protected *in vitro* is in the viral strand (nucleotides 33–69), this fragment could represent an *in vivo* ribosome binding site. The sequence implicated in ribosome binding (nucleotides 42–46) is in the expected relationship to an initiation codon (ATG, nucleotides 54–56)[14]. The adjacent DNA sequence allows the prediction of the sequence of the N-terminal 21 amino acids of the presumed protein product.

It is not possible unequivocally to assign this ribosome binding site, and the putative protein, to a particular gene. The limits of genes *H* and *D* are known in relation to the physical map of ΦX174 (ref. 1 and G. M. Air, A. R. Coulson F. Sanger, personal communication). The region between genes *H* and *D* contains genes *A*, *B* and *C*. In addition, a

Table 1 Observed molar yields of depurination products

Primer/template	*Hin*fI 6/viral		*Hin*dII 8/complementary	
Label	dATP	dGTP	dATP	dGTP
Depurination products				
T	2	1	1	2
C	>3 (4)	1	2	
T_2		1		
C T			>3 (5)	>3 (4)
C_2			1	
C T_2		2	2	
C_2T	1		1	
C T_3		1		
C_2T_2	1			1
T_5	1			
C_2T_3	1			
C_3T_2	1			
C T_5	1	1		
C_2T_4		1*		
C_4T_2			1	

Only the depurination products predicted from the gel sequence data were observed, and in their predicted molar yields. It was not possible to quantitate the products present in greater than three molar yield, and the predicted yields for these are given in parentheses.

*This depurination product established the identity of nucleotide 97 (A, Fig. 4) which was not identified by gel sequence data.

smaller protein (the A* protein) is produced by an internal translational start within gene *A* (ref. 15). The genetic evidence[7,16] and the size of the A protein[19] place the start of gene *A* close to the end of gene *H*. The size of the A* protein[17] and the genetic data[16] would allow its start in the general region of the new ribosome binding site. This assignment is, however, excluded by the termination codons, TAA, at nucleotides 3, 33 and 48 immediately in front of, and in phase with, the ATG at nucleotide 54.

Gene *C* maps a considerable distance to the right of the new ribosome binding site[7] and can therefore be excluded from consideration. The estimates of the size of the gene *B* protein[18–20] are compatible with the assignment of the site to gene *B*. The genetic data[7] places the start of gene *B* to the right of this site, within *Alu*I fragment 5. However, mutants in this region assigned to genes *A* or *B* behave aberrantly in reversion studies (C. A. Hutchison, personal communication) and the genetic data cannot therefore exclude the assignment of the site to gene *B*. A class of mutants, exemplified by *to*8, has been mapped within *Alu*I fragment 12a, and has therefore been assigned to gene *A* (ref. 21). This class may define a new cistron[21], however, and the ribosome binding site is compatible with such a cistron. An alternative possibility is that these mutants should be assigned to gene *B*.

In addition to the termination signals in the same phase as the initiation codon at nucleotide 54, this region of DNA contains two termination codons at nucleotides 41 (TAG) and 68 (TAA). Both of these are in a second phase. There are no termination signals in this region in the third phase. Therefore, the possibility of two overlapping genes, analogous to genes *D* and *E* (ref. 1), cannot be excluded. Such an overlap may help overcome the problem of fitting genes *A*, *B* and *C* (whose sizes[1] are calculated from the estimated molecular weights of their gene products[17–20,22]) into the available DNA, and may explain some of the paradoxes presented by the genetic data[7,21].

Determination of the N-terminal amino acid sequence of the B protein and any other likely gene products, and their comparison with the 21 amino acid sequence predicted from the DNA sequence data, should allow unequivocal assignment of this ribosome binding site. Further knowledge of the functions of this region of the genome will probably result from a more extensive DNA sequence determination on either side of the sequence presented here, and from examination of the DNA sequence in genetically defined mutants. The results of these studies are presented in ref. 25.

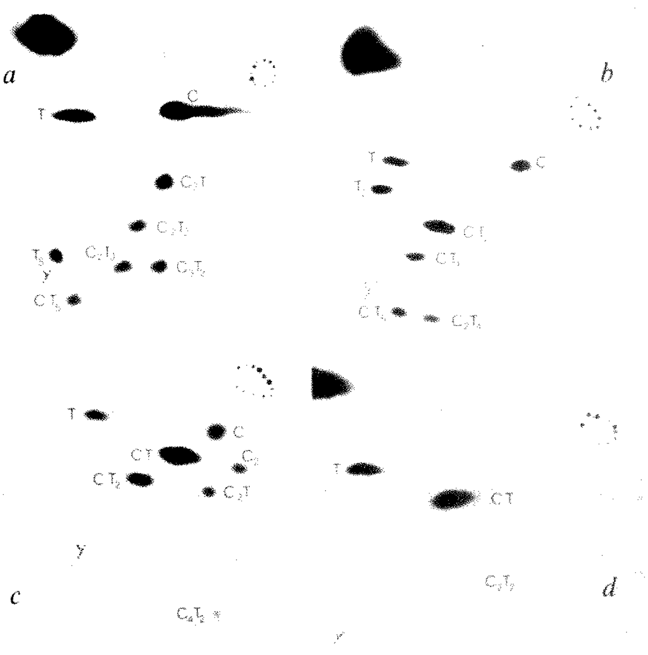
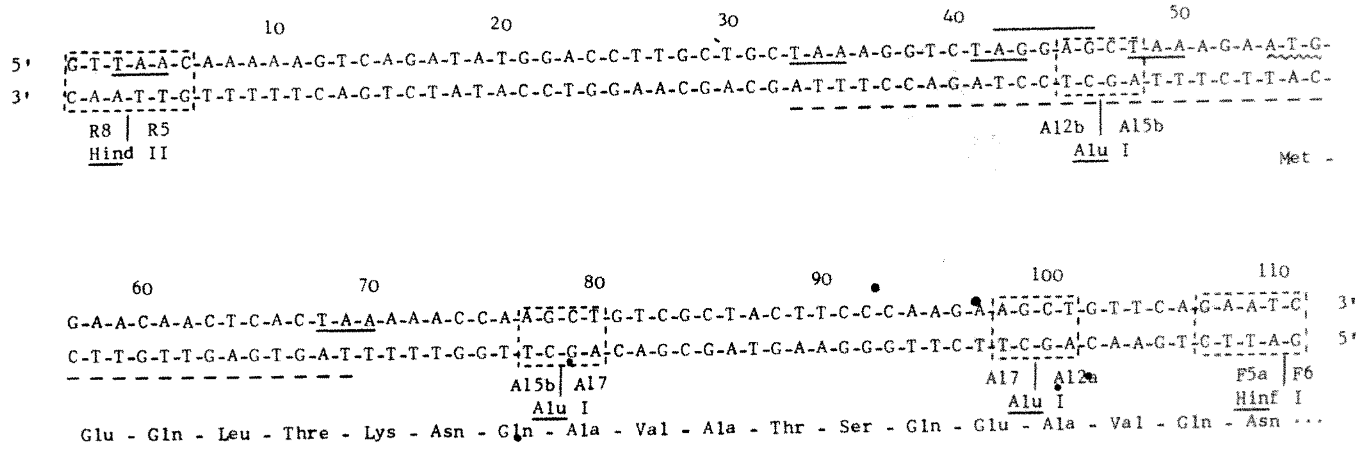


Fig. 3 Depurination analysis. The pyrimidine tracts in each strand were terminally labelled and isolated by the method of Fiddes[10]. *Hinf*I fragment 6 was used as a primer on viral strand DNA as template for primed synthesis with either α-^{32}P-dATP or α-^{32}P-dGTP label in separate experiments. *Hin*dII fragment 8 was similarly used as a primer on complementary strand DNA. The region between the *Hin*dII 8/5 and *Hinf*I 5a/6 cleavage sites was isolated by cleavage with both enzymes, followed by electrophoresis of the products on a gel containing 5% acrylamide, 2.5 mM EDTA, 90 mM Tris-borate (*p*H 8.3). The purified fragment was eluted in 0.5 M NaCl, 0.005 M EDTA, 0.1 M Tris-Cl (*p*H 8.5) and ethanol-precipitated. Depurination of the fragment and separation of the products were according to the methods of Ling[11]. As the products were end-labelled by nearest-neighbour transfer of ^{32}P from the adjacent purine, they could be quantitated by comparison of the relative intensities of the spots on the autoradiogram. The quantitative data is given in Table 1. *a*, *Hinf*I fragment 6, viral strand template, α-^{32}P-dATP label (that is, complementary strand depurination products). *b*, as (*a*), with α-^{32}P-dGTP label. *c*, *Hin*dII fragment 8, complementary strand template, α-^{32}P-dATP label (that is, viral strand depurination products). *d*, as (*c*), with α-^{32}P-dGTP label.

Fig. 4 The sequence of ΦX174 *am*3 RF DNA from the *Hin*dII 8/5 cleavage site to the *Hinf*I 5a/6 cleavage site. Plus and minus sequence determination priming with *Hinf*I fragment 6 on viral DNA template gave the sequence from 1 to 94. Priming with *Hin*dII fragment 8 on complementary DNA template gave the sequence 19–111. Thus most of the sequence is confirmed by complementary data, including the region corresponding to the ribosome protected RNA fragment (33–69; – – –). Nucleotide 97 (A) was established solely by depurination analysis. Termination codons are underlined, and restriction endonuclease cleavage sites are boxed. The presumed initiation codon is underlined with a wavy line. The sequence implicated in ribosome binding (AGGAG) is overlined.



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              10                            20                            30                            40                            50
5'  G-T-T-A-A-C-A-A-A-A-A-G-T-C-A-G-A-T-A-T-G-G-A-C-C-T-T-G-C-T-G-C-T-A-A-A-G-G-T-C-T-A-G-G-A-G-C-T-A-A-A-G-A-A-T-G-
3'  C-A-A-T-T-G-T-T-T-T-T-C-A-G-T-C-T-A-T-A-C-C-T-G-G-A-A-C-G-A-C-G-A-T-T-T-C-C-A-G-A-T-C-C-T-C-G-A-T-T-T-C-T-T-A-C-
      R8 | R5                                                                                       A12b | A15b
      Hind II                                                                                          Alu I                Met -

       60                       70                       80                       90                  100                     110
G-A-A-C-A-A-C-T-C-A-C-T-A-A-A-A-A-C-C-A-A-G-C-T-G-T-C-G-C-T-A-C-T-T-C-C-C-A-A-G-A-A-G-C-T-G-T-T-C-A-G-A-A-T-C   3'
C-T-T-G-T-T-G-A-G-T-G-A-T-T-T-T-T-G-G-T-T-C-G-A-C-A-G-C-G-A-T-G-A-A-G-G-G-T-T-C-T-T-C-G-A-C-A-A-G-T-C-T-T-A-G   5'
                                      A15b | A17                                 A17 | A12a                  F5a | F6
                                         Alu I                                      Alu I                    Hinf I
  Glu - Gln - Leu - Thre - Lys - Asn - Gln - Ala - Val - Ala - Thr - Ser - Gln - Glu - Ala - Val - Gln - Asn ...
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Isolation and characterisation of the ΦX174 ribosome binding sites

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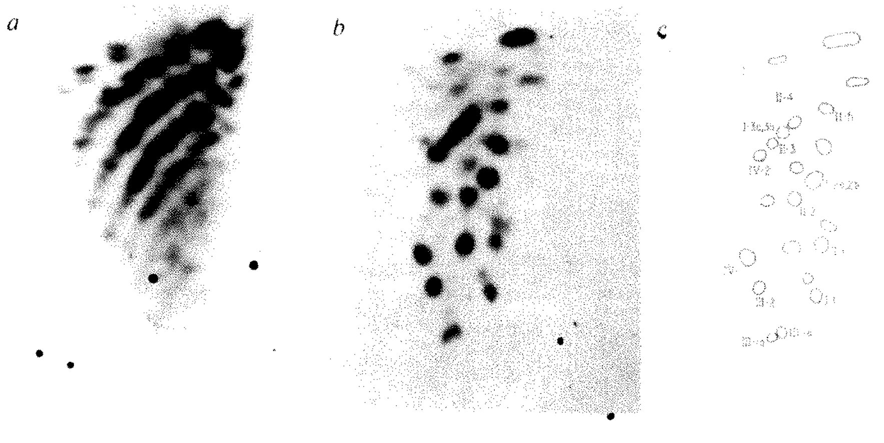
Sequence information for four RNA ribosome binding sites has been obtained from ΦX174 ΦRNA synthesised in vitro. *The ribosome binding sites have been assigned to sites on the ΦX174 genome by comparison with known DNA sequence data.*

ΦX174 is a single-stranded DNA bacteriophage with a genome of approximately 5,400 nucleotides which codes for nine products (for a review see ref. 1). In the infected cell the mRNA is transcribed from a double-stranded DNA replicative form and translated into the phage proteins. *In vivo*, the proteins made in largest amount are the products of genes *D*, *F* and *G* (refs 2 and 3). Robertson *et al.*[4-6] isolated a ΦX174 DNA fragment protected by ribosomes which contained a DNA sequence coding for the amino terminal portion of the gene *G* protein. In this laboratory, Pieczenik *et al.*[7] isolated and determined the sequences of three ribosome binding sites from the mRNA of bacteriophage f1 using techniques that have previously been shown to yield authentic protein initiation sites from the RNA bacteriophages[8-10].

We now have isolated and obtained sequence information for four RNA ribosome binding sites derived from ΦX174 mRNA synthesised *in vitro*. In an accompanying paper, Brown and Smith[11] report the DNA sequence of a ΦX174 region which includes one of these sites and thereby locates this site on the ΦX174 genome. Recently, Barrell *et al.*[12] have reported the DNA sequences encoding the amino termini of ΦX174 proteins D, J, F, G and H. Comparison of the DNA and the amino acid sequence data for these ΦX174 proteins allowed them to make these assignments.

Availability of these and other DNA sequence data[4-6,12] has allowed us to assign the other three ribosome binding sites to ΦX174 genes and to propose complete sequences for them. Our studies of the interaction of ΦX174 mRNA with ribosomes complement the DNA sequence data and allow us to define regions of the genome encoding signals for initiation of protein synthesis.

Fig. 1 Two-dimensional RNase T_1 fingerprint analysis of ΦX174 RNA transcripts before (*a*) and after (*b*) ribosome binding. *c*, Major oligonucleotides of the four ribosome binding sites (I, II, III and IV). Sequence analysis of these oligonucleotides is found in the text and Figs 3, 4 and 5. mRNA transcripts were prepared from ΦX174 RFI and in the experiment shown α-^{32}P-ATP at 130 Ci mmol^{-1} was used to prepare the material analysed here. Conditions of transcription and purification of transcripts are as described previously[21]. Ribosome binding was done as described by Steitz[9] and the fragments isolated and purified as described by Pieczenik *et al.*[7] Two-dimensional fingerprint analysis was carried out according to Barrell[22]. The origin is located in the lower right corner of the autoradiograph with electrophoresis at *p*H 3.5 on cellulose acetate proceeding from right to left and homochromatography from bottom to top on DEAE thin-layer plates.



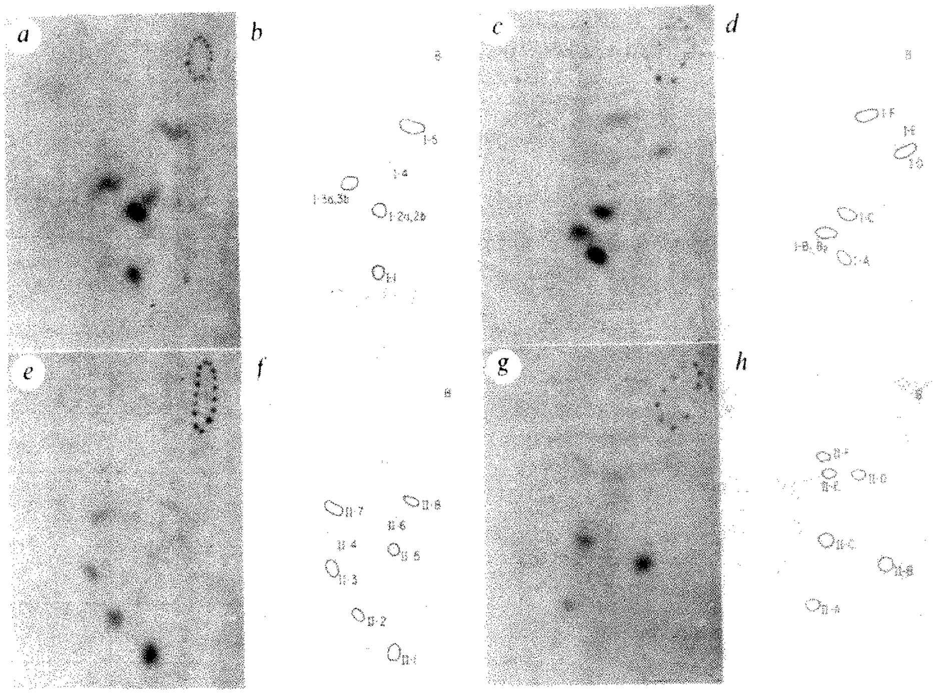


Fig. 2 RNase T_1 and pancreatic RNase fingerprinting analysis of the two ribosome binding site fragments purified by two-dimensional homochromatography. *a* (drawn in *b*) shows the RNase T_1 fingerprint of site I, while *c* (drawn in *d*) shows the pancreatic RNase fingerprint of site I. Site II is shown in *e–h* with the results of RNase T_1 digestion in *e* (drawn in *f*) and pancreatic RNase digestion in *g* (drawn in *h*). Sequence analysis of the oligonucleotides indicated here is discussed in the text and in Figs 3 and 4. Dotted circles represent spots not seen when α-^{32}P-ATP was used to synthesise mRNA, but found in analogous fingerprints of these sites using other α-^{32}P precursors. The origin is in the lower right-hand corner. B denotes blue dye mark. Details of the purification and fractionation of RNA ribosome binding site fragments using this approach are to be found in Pieczenik *et al.*[7]

Isolation of ribosome binding sites

ΦX174 RFI DNA was transcribed *in vitro* in the presence of α-^{32}P-nucleoside triphosphates to obtain labelled mRNA. Ribosomes were bound to these transcripts in conditions described previously[9] and the initiation complexes were digested with pancreatic RNase. Between 0.2 and 0.5% of the input mRNA was protected from RNase digestion and sedimented with 70S ribosomes in these conditions. Figure 1 shows two-dimensional RNase T_1 fingerprint analysis of ΦX174 mRNA (*a*) and its ribosome-protected fragments (*b*). Panel (*c*) identifies the major RNase T_1-resistant oligonucleotides (T_1-oligonucleotides) obtained from the unfractionated mixture of ribosome-protected fragments. Figure 1 shows that protection of ΦX174 mRNA by ribosomes results in a considerable simplification of the T_1-oligonucleotide pattern.

To obtain homogeneous ribosome-protected fragments for further sequence analysis, the mixture of fragments was fractionated using homochromatography as the second dimension of a two-dimensional system[13], yielding two purified ribosome binding site species (data not shown). Figure 2 shows RNase T_1 and pancreatic RNase fingerprinting analysis of ribosome binding site I (*a–d*) and ribosome binding site II (*e–h*). Experiments were carried out with mRNA labelled by each of the four α-^{32}P-ribonucleoside triphosphates individually, or labelled by all four triphosphates. Nearest neighbour sequence analysis was exploited to generate the sequence of each oligonucleotide, as well as to determine the order of several of the oligonucleotides[7].

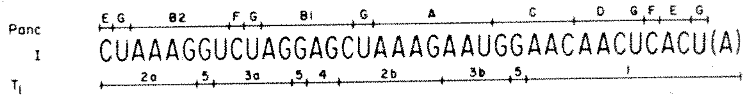
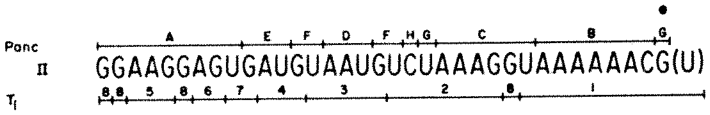


Fig. 3 Sequence analysis of ribosome binding site I. Conditions for base composition and nuclease digestion are according to Barrell[22]. NTP refers to the α-^{32}P-ribonucleoside triphosphate precursor used to synthesise the oligonucleotides analysed in a given line. N.D. means that the oligonucleotide in question was obtained but the analysis was not done, and a dash signifies that the particular oligonucleotide was not present. The sequence shown at the top of the table is the only arrangement of T_1 (shown by the number below the sequence) and panc oligonucleotides (shown by the letters above the sequence) consistent with the data shown here. *Nucleotides found to be labelled after base composition analysis. ‡RNase U_2 occasionally incomplete digestion products and yielded undigested starting material.

PROPOSED SEQUENCE	NTP	COMPOSITION	PANCREATIC RNase PRODUCTS	RNase U_2 PRODUCTS
1 AACAACUCACU(A)	A	2C,2A,1U	A*AC,A*AC‡C‡U*	CUC*A,C*A‡CU‡A*A,A*
	U	C	AAC*,AC*	C*UCA,C*U
	C	A	AA*C,A*C,U*	CU*CA*,AA*,A*
	G	-	-	-
	A,C	N.D.	AAC,AC,U,C	N.D.
	A,U,C,G	A,C,U	AAC,AC,C,U	N.D.
2a CUAAAG(G)	A	2A,1U	A*A*AG,U*	CU*A*A‡CU*A‡A*A,A*
	U	C	C*	C*UA
	C	-	-	-
	G	A,G	AAA*G*	N.D.
	A,C	N.D.	A*A*AG,U*	N.D.
	A,U,C,G	C,A,G,U	AAAG,C,U	N.D.
2b CUAAAG(A)	A	2A,1G,1U	A*A*AG*,U*	CU*A*A‡CU*A‡A*A,A‡G*
	U	C	C*	C*UA
	C	-	-	-
	G	A	AAA*G	AA*,A*
	A,C	N.D.	A*A*AG*,U	N.D.
	A,U,C,G	C,A,G,U	AAAG,C,U	N.D.
3a UCUAG(G)	A	U	U*	UCU*A
	U	C	C*	UC*UA
	C	U	U*	U*CUA
	G	A,G	A*G*	UCUA*
	A,U,C,G	C,A,G,U	AG,C,U	N.D.
3b AAUG(G)	A	A	A*AU	A*A,A*
	U	A	AA*U	AA*,A*
	C	-	-	-
	G	G,U	AAU*,G*	U*G*
	A,U,C,G	A,G,U	AAU,G	N.D.
4 AG(C)	A	-	-	-
	U	-	-	-
	C	G	AG*	AG*,G*‡
	G	A	A*G	A*C,A*‡
	A,C	N.D.	AG	N.D.
	A,U,C,G	A,G	AG	N.D.
5 G(U/A)	A	G	G	G
	[illegible]	G	G	C
	C	-	-	-
	G	-	-	-
	A,C	N.D.	G	N.D.
	A,U,C,G	G	G	N.D.

PROPOSED SEQUENCE	NTP	COMPOSITION	RNase T_1 PRODUCTS
A AAAGAAU(G)	A	3A,1G	A*A*AG*,A*AU
	U	A	AA*U
	C	-	-
	G	A	AAA*G,AAU*
	A,C	N.D.	A*A*AG*,A*AU
B_1 AGGAGC(U)	A	G	G*
	U	C	C*
	C	G	AG*
	G	2A,G	A*G*,A*G
	A,C	N.D.	AG*,G*
B_2 AAAGGU(C)	A	A	A*A*AG
	U	G	G*
	C	U	U
	G	A,G	AAA*G*
	A,C	N.D.	A*A*AG,U*
C GGAAC(A)	A	G,A,C	G*,A*AC*
	U	-	-
	C	A	AA*C
	G	G	G*
	A,C	N.D.	A*A*C*,G*
D AAC(U)	A	A	A*AC
	U	C	AAC*
	C	A	AA*C
	G	-	-
	A,C	N.D.	A*A*C
E AC(U)	A	-	-
	U	C	AC*
	C	A	A*C
	G	-	-
	A,C	N.D.	A*C
F C(A/U)	A	C	C
	U	C	C
	C	-	-
	G	-	-
	A,C	N.D.	C
G U(A/C)	A	U	U
	U	-	-
	C	U	U
	G	-	-
	A,C	N.D.	U



PROPOSED SEQUENCE	NTP	COMPOSITION	PANC/T_1	U_2/T_1
1 UAAAAAACG(U)†	A	>4A, 1U	A_n^*C, U*	U*A*, A*A*, A*
	U	N.D.	N.D.	N.D.
	C	A	N.D.	N.D.
	G	C	A_n^*C	C*G
	A,C	N.D.	A_n^*C, U*	N.D.
	A,U,C,G	C,A,G,U	A_nC,G,U	CG,A
2 UCUAAAG(G)	A	2A, 1U	A*A*AG,U*	UCU*A*A*A, A*
	U	C	C*	UC*UA
	C	U	U*	U*CUA
	G	A,G	AAA*G*	AA*,G
	A,C	N.D.	A*A*AG,U*	N.D.
	A,U,C,G	C,A,G,U	AAAG,U,C	UCUA,A,G
3 UAAUG(U)	A	A,U	A*AU,U*	U*A*
	U	A,G	AA*U,G*	UG*,A*
	C	-	-	-
	G	U	AAU*	U*G
	A,C	N.D.	A*AU,U	N.D.
	A,U,C,G	A,G,U	AAU,G,U	N.D.
4 AUG(U)	A	-	-	-
	U	A,G	A*U,G*	UG*,A*
	C	-	-	-
	G	U	AU*	U*G
	A,U,C,G	A,G,U	AU,G	N.D.
5 AAG(G)	A	A	A*AG	A*A,A*
	U	-	-	-
	C	-	-	-
	G	A,G	AA*G	AA*,G*,A*
	A,U,C,G	A,G	AAG	N.D.
6 AG(U)	A	-	-	-
	U	G	AG*	AG*,G*
	C	-	-	-
	G	A	A*G	A*G,A*
	A,U,C,G	A,G	AG	N.D.
7 UG(A)	A	G	G	UG*
	U	-	-	-
	C	-	-	-
	G	U	U	U*G
	A,U,C,G	U,G	G,U	N.D.
8 G(G/A/U)	A	G	G	G
	U	G	G	G
	C	-	-	-
	G	G	G	G
	A,U,C,G	G	G	N.D.

PROPOSED SEQUENCE	NTP	COMPOSITION	T_1/PANC
A GGAAGGAGU(G)	A	2G, 1A	A*AG,G*
	U	G	AG*
	C	-	-
	G	2G, 2A, 1U	AA*G,A*G,G*,U*
	A,C	N.D.	A*AG,G*
	A,U,C,G	N.D.	AAG,AG,G,U
B AAAAAAC(G)†	A	A	A_n^*C
	U	-	-
	C	N.D.	A_n^*C
	G	C	A_nC^*
	A,C	N.D.	A_n^*C
	A,U,C,G	N.D.	A_nC
C AAAGGU(A)	A	2A,U	A*A*AG,U*
	U	G	G
	C	-	-
	G	A,G	AAA*G*
	A,C	N.D.	A*A*AG,U
	A,U,C,G	N.D.	AAAG,G,U
D AAU(G)	A	A	A*AU
	U	A	AA*U
	C	-	-
	G	U	AAU*
E GAU(G)	A	G	G
	U	A	A*U
	C	-	-
	G	U	AU*
	A,C	N.D.	G
F GU(A/C)	A	U	U
	U	G	G
	C	U	U
	G	-	-
G U(A)	A	U	U
	U	-	-
	C	-	-
	G	-	-
H C(U)	A	-	-
	U	C	C
	C	-	-
	G	-	-
	A,C	-	-

Fig. 4 Sequence analysis of ribosome binding site II. The ordering of T_1 oligonucleotides 1, 2 and 3 is dependent on the DNA sequencing data of Barrell *et al.*[12]. Other details are as in the legend to Fig. 3. †The data obtained do not specify the value of n beyond the fact that $n > 4$. The actual value of n is derived from the DNA sequence data of Barrell *et al.*[12].

Sequence analysis of binding site I

The nucleotide sequence of binding site I was determined. Figure 3 shows the T_1 and pancreatic RNase-resistant oligonucleotides (panc oligonucleotides) obtained from the purified species shown in Fig. 1 (*a–d*). T_1 oligonucleotides 2a and 2b were present as a mixture and could be distinguished by nearest neighbour labelling patterns. The mixture of T_1 oligonucleotides 3a and 3b was resolved into two components by electrophoresis on DEAE paper in 7% formic acid (data not shown). Panc oligonucleotides B_1 and B_2 were distinguished by their nearest neighbour labelling pattern. In binding site I, the panc oligonucleotides allow us to order the T_1 oligonucleotides yielding the sequences we have written.

The single base overlap of T_1 oligonucleotides 4 and 2b generated by panc oligonucleotide B_1 is unambiguous since T_1 oligonucleotide 4, AG, is uniquely labelled by a C nearest neighbour, a feature shared by no other T_1 oligonucleotide in Fig. 3. This establishes that AG must be located 5′ to a T_1 oligonucleotide whose 5′-end is C. T_1 oligonucleotide 2b, CUAAAG(A), has been overlapped with T_1 oligonucleotide 3b by panc oligonucleotide A and is the only candidate which can label AG by C. We can therefore write the sequence . . . AGC(U), a sequence that can only come from panc oligonucleotide B_1, establishing the sequence as we have written it.

Sequence analysis of other ribosome binding sites

The data substantiating the other purified ribosome binding site fragment shown in Fig. 2 (*e–h*), are summarised in Fig. 4. The sequence written relies upon the DNA sequence data obtained by Barrell *et al.*[12] to determine the value of n in T_1 oligonucleotide 1, $A_nCG(U)$ and to order the T_1 oligonucleotides 1, 2 and 3.

We have obtained and sequenced additional T_1 and panc oligonucleotides from the mixture of unfractionated ΦX174 ribosome binding sites (see Fig. 5). We were unable to purify the individual ribosome binding sites to which these could be assigned. Thus the overlaps shown in Fig. 5 were derived from a comparison of these oligonucleotide sequences with DNA sequence data[4–6,12].

The sequences of the four ΦX174 ribosome binding sites are shown in Fig. 6, with their predicted amino acid sequences as specified by the codons following the AUG.

The assignment of these RNA ribosome binding sites to ΦX174 genes has been possible as a result of the DNA sequence information accumulated for ΦX174 by Brown and Smith[11], Barrell *et al.*[12] and Robertson *et al.*[4–6]. Site I is believed to correspond to the initiation site for gene *B*, on the basis of the data of Brown and Smith[11], whose independent sequence analyses have assigned this sequence to restriction fragments mapping within gene *B*. Site II has been identified as the gene *J* initiation site on the strength of the DNA and protein sequence information obtained for that region of the genome (ref. 12 and B. G. Barrell, in preparation). Similarly, site III can be assigned to the gene *F* initiation site[12,14,15] and site IV has been identified as the gene *G* initiation site[4–6].

ΦX174 specifies nine viral gene products. As in the case of f1 (ref. 7), the *in vitro* ribosome protection method does not yield mRNA fragments corresponding to each potential protein initiation site. Rather, a subset of potential sites is protected from nuclease digestion. In addition, in the case of ΦX174 Robertson *et al.*[4–6] have shown that a single predominant species, derived from gene *G*, is protected from DNase digestion when ΦX174 single-stranded viral DNA is bound to ribosomes. We have found that site IV (gene *G*) is not consistently isolated as an RNA ribosome binding site and when present is seen in the lowest yield of the four sites described here. We have found no obvious correlation between the recovery of any specific ribosome binding site and the amount of the corresponding protein synthesised either *in vivo*[2,3] or *in vitro*[16].

Recently, Shine and Dalgarno[17] have proposed that the 3′-end of 16S rRNA, 5′-GAUCACCUCCUUA$_{OH}$, is able to form Watson–Crick base pairs with sequences within the RNA ribosome binding sites, stabilising protein initiation sites in ribosome–mRNA complexes. That such base pairs can, in fact, be formed was demonstrated directly by Steitz and Jakes[18]. It has been shown, however, that an intact 3′-end of 16S rRNA

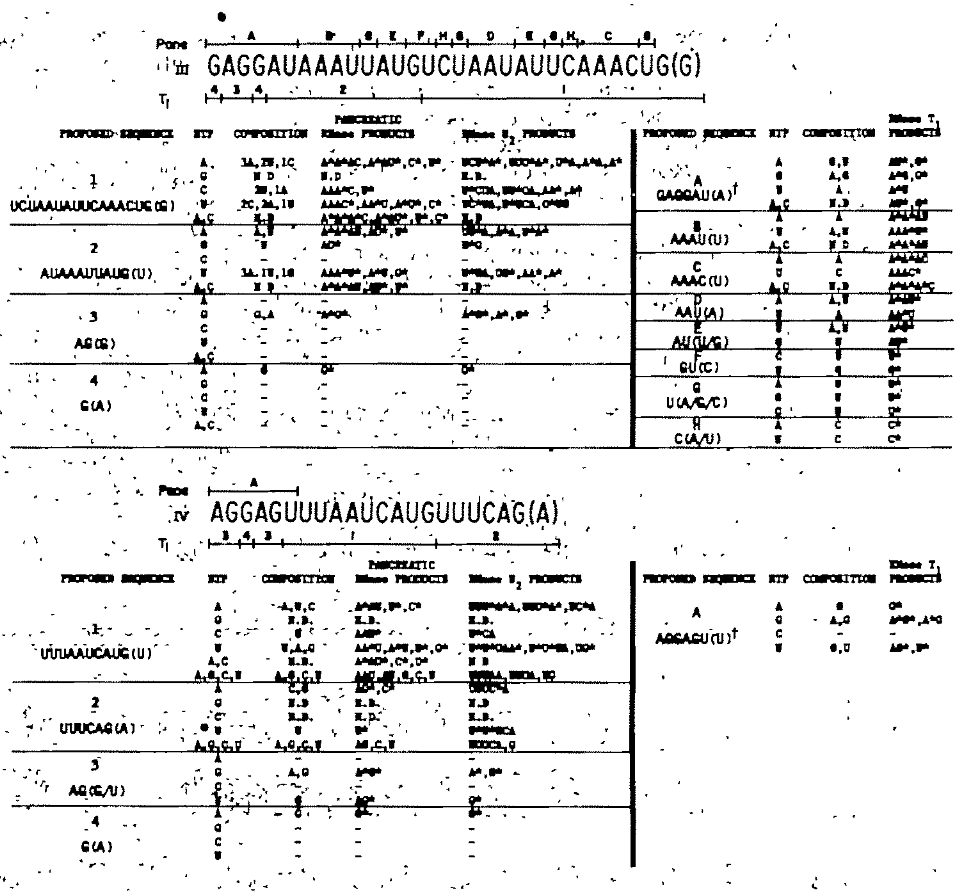

Pos
III GAGGAUAAAUUAUGUCUAAUAUUCAAACUG(G)
T1
PROPOSED SEQUENCE
RIP
COMPOSITION
PANCREATIC RNase PRODUCTS
RNase U2 PRODUCTS
UCUAAUAUUCAAACUG(G)
AUAAAUUAUG(U)
AG(G)
G(A)
GAGGAU(A)†
AAAU(U)
AAAC(U)
AAU(A)
AU(U/G)
GU(C)
U(A/G/C)
C(A/U)
RNase T1 PRODUCTS
IV AGGAGUUUAAUCAUGUUUCAG(A)
UUUAAUCAUG(U)
UUUCAG(A)
AG(G/U)
G(A)
AGGAGU(U)†

Fig. 5 Sequence analysis of binding sites III and IV. Details of the sequence analysis are described in the text and the legend to Fig. 3. †GAGGAU and AGGAGU were isolated as a mixture and their individual sequences deduced by nearest neighbour sequence analysis.

is not required to form stable initiation complexes *in vitro*[10]. Indeed, in all ribosome binding sites sequenced to date, such potential complementarity to the 3′-end of 16S rRNA has been found to exist. The four ribosome binding sites reported here all possess sequences that have the potential to form base-paired complexes with the 3′-end of 16S rRNA. Thus, the sequence 5′ . . . AGGAG . . . 3′ present in site I has the potential to form five such base pairs, site II can form a possible ten base-paired complex (if a single base is looped out of the 3′-end of 16S rRNA), while sites III and IV can each form a possible five base pairs. The same base-pairing schemes for the DNA sequences encoding sites II, III and IV have been proposed by Barrell *et al.*[12], along with similar schemes for genes *D*, *E* and *H*. We can find no correlation between the number of potential Shine–Dalgarno base pairs seen in an initiation site and the efficiency of ribosome binding and protection *in vitro*. Clearly, other factors must determine which of the protein initiation sites are selected and recognised by ribosomes in our *in vitro* system.

Brown and Smith[11], in an accompanying paper present evidence that site I may be the gene *B* ribosome binding site. Their data suggest that the gene *B* protein is encoded by the DNA sequence which also codes for the carboxy-terminus of the gene *A* protein. Barrell *et al.*[12] have shown that gene *E* is similarly coded for by the DNA sequence which also codes for the gene *D* protein. We have found no evidence for a ribosome binding site *in vitro* for either gene *D* or gene *E*. Thus the transcripts from one of the 'included' genes are efficiently protected by ribosomes while those from the other are not.

Binding site II, the proposed gene *J* binding site, contains the sequence 5′-GGAAGGAG$\overline{\text{UGA}}$UG$\overline{\text{UAA}}$UG . . . 3′. The indicated UGA codon would terminate the proposed gene *E* protein[12], whereas the indicated *D* termination codon (UAA) overlaps the indicated gene *J* initiation codon (AUG) by a single base. Platt and Yanofsky[20] have observed a similar one-base overlap between the termination of one gene and the initiation of a second gene in the tryptophan operon. Here two termination signals and one functional initiation signal are compressed within ten bases showing that large intercistronic spaces are not required to separate signals for gene expression.

Homologies between published ribosome binding site sequences have been reported. Here we find that sites III (gene *F*) and IV (gene *G*) have a seven out of eight base homology to the left of and including the AUG. In addition, site IV (gene *G*) has six bases (UUUAAU) in common with f1 binding site 1 (gene *VIII*) (ref. 7) and nine out of ten bases from the same region in common with the Qβ coat protein ribosome binding site[10]. Further analysis will be required to assess the significance of these homologies.

Finally, it should be noted that site IV (gene *G*) is considerably smaller when protected as an mRNA species in a ribosome binding complex than when the single-stranded viral DNA is used in the *in vitro* binding system. Robertson *et al.*[4–6] sequenced a 49-nucleotide protected fragment. We find the fragment which is identified as the gene *G* ribosome binding site has a sequence of only 21 nucleotides. The 14 nucleotide-long

I 5′GCUAAAGGUCUAGGAGCUAAAGAAUGGAACAACUCACU(A)3′
Met—Glu—Gln—Leu—Thr

II 5′GGAAGGAGUGAUGUAAUGUCUAAAGGUAAAAAACG(U)3′
Met—Ser—Lys—Gly—Lys—Lys—Arg

III 5′GAGGAUAAAUUAUGUCUAAUAUUCAAACUG(G)3′
Met—Ser—Asn—Ile—Gln—Thr

IV 5′AGGAGUUUAAUCAUGUUUCAG(A)3′
Met—Phe—Gln

Fig. 6 Proposed sequences of the four RNA ribosome binding sites of ΦX174. Details of the sequence analysis are described in the text and Figs 3, 4 and 5. The nucleotide in parentheses at the 3′-end of each sequence represents the nearest neighbour nucleotide determined for the 3′-terminal RNase T_1 digestion product in each case. Binding site I predicts an amino terminal sequence for the gene *B* protein (see text and the accompanying paper). Binding sites II, III and IV specify the amino termini of genes *J*, *F* and *G* (refs 12 and 14–16).

T_1 oligonucleotide on the 3′-end of the DNA sequence was not recovered, nor was a seven nucleotide-long T_1 oligonucleotide on the 5′-end of the DNA sequence. Both of these T_1 oligonucleotides contain extensive polypyrimidine tracts which might explain the poor recovery of these oligonucleotides in the conditions of pancreatic RNase digestion used here to isolate ribosome protected fragments.

We have carefully compared the sequences of these four ribosome binding sites with the six potential ribosome binding sites described by Barrell *et al.*[12] but are as yet unable to explain why only four are recovered in this *in vitro* system. Those which are recovered allow us to define, in conjunction with the accompanying paper and earlier studies[4–6,11], additional functional regions of the ΦX174 DNA sequence.

We thank Bart Barrell, Nigel Brown and Mike Smith for generously providing DNA sequencing data in advance of publication. We further thank Bart Barrell for helpful suggestions, and George Pieczenik and Norton Zinder for encouragement, advice and suggestions. We thank Edward Pelle and Carolyn McGill for technical assistance. This research was supported in part by grants from the US NFS and the US National Institutes of Health.

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DNA sequence at the C termini of the overlapping genes *A* and *B* in bacteriophage ΦX174

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Sequence determination of mutants in genes A *and* B *confirms that these constitute another pair of genes in ΦX174 which are translated from the same DNA sequence using different reading frames.*

BACTERIOPHAGE ΦX174 is known to code for nine proteins in its DNA (ref. 1) which is about 5,400 nucleotides in length (Fig. 1). The locations of the boundaries of six of the genes—*D*, *E*, *J*, *F*, *G* and *H*—have been defined by sequence determination (F.S. *et al*, in preparation). Two of these genes (*D* and *E*) overlap on the DNA sequence[1]. To define the boundaries for genes *A*, *B* and *C* which lie in the remaining region between gene *H* and gene *D*, the sequence of ΦX174 *am*3 DNA in the region between the *Hae*III 6a/6b and the *Taq*I 2/7 sites (Fig. 1) has been determined (N.L B. and M.S., in preparation). A newly recognised ribosome binding site[3] has been located in the sequence[4]. The sequence determination of a *B* gene mutant—*am*16—strongly suggested that the *A* and *B* genes are translated from the same DNA using different reading frames (N.L.B. and M.S., in preparation). We now report the 200 nucleotide sequence of ΦX174 *am*3 DNA from the *Taq* 2/7 to the *Hha* 8b/4 sites (Fig. 1). In addition, the sequences of three mutants from this region—*am*18 and *am*35 (gene *A*) and a revertant of *am*18, *ts*116 (gene *B*)—have been determined. The mutants *am*18 and *am*35 have an

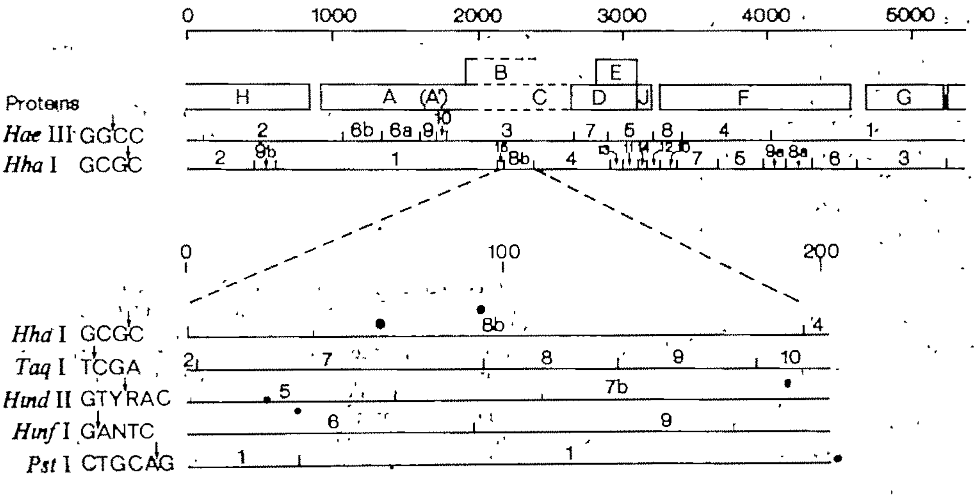


Fig. 1 Map of the ΦX174 genome showing the *Hae*III and *Hha*I fragments and the protein-coding sequences and details of other restriction cleavages within *Hha* fragment 8b.

amber codon at the same site. The gene *B* mutant, *ts*116, results from a single base change in the termination codon of *am*18/35. These results confirm the overlap of genes *A* and *B* and establish the C-termini of the two genes.

Nucleotide sequence analysis

The cleavage sites of five restriction enzymes which cleave in the region are shown in Fig. 1. The sequence of the DNA was obtained by a slight modification of the 'plus and minus' method (ref. 5 and N.L.B. and M.S., in preparation) by priming from the *Hin*fI 6/9 site, on both viral and complementary templates and from the *Pst*I site on the complementary template (Fig. 2). The sequence was confirmed by depurination analysis[6] of *Taq*I fragments 7, 8, 9 and 10 labelled by 'nick translation'[7] of ΦX174 *am*3 RFI DNA.

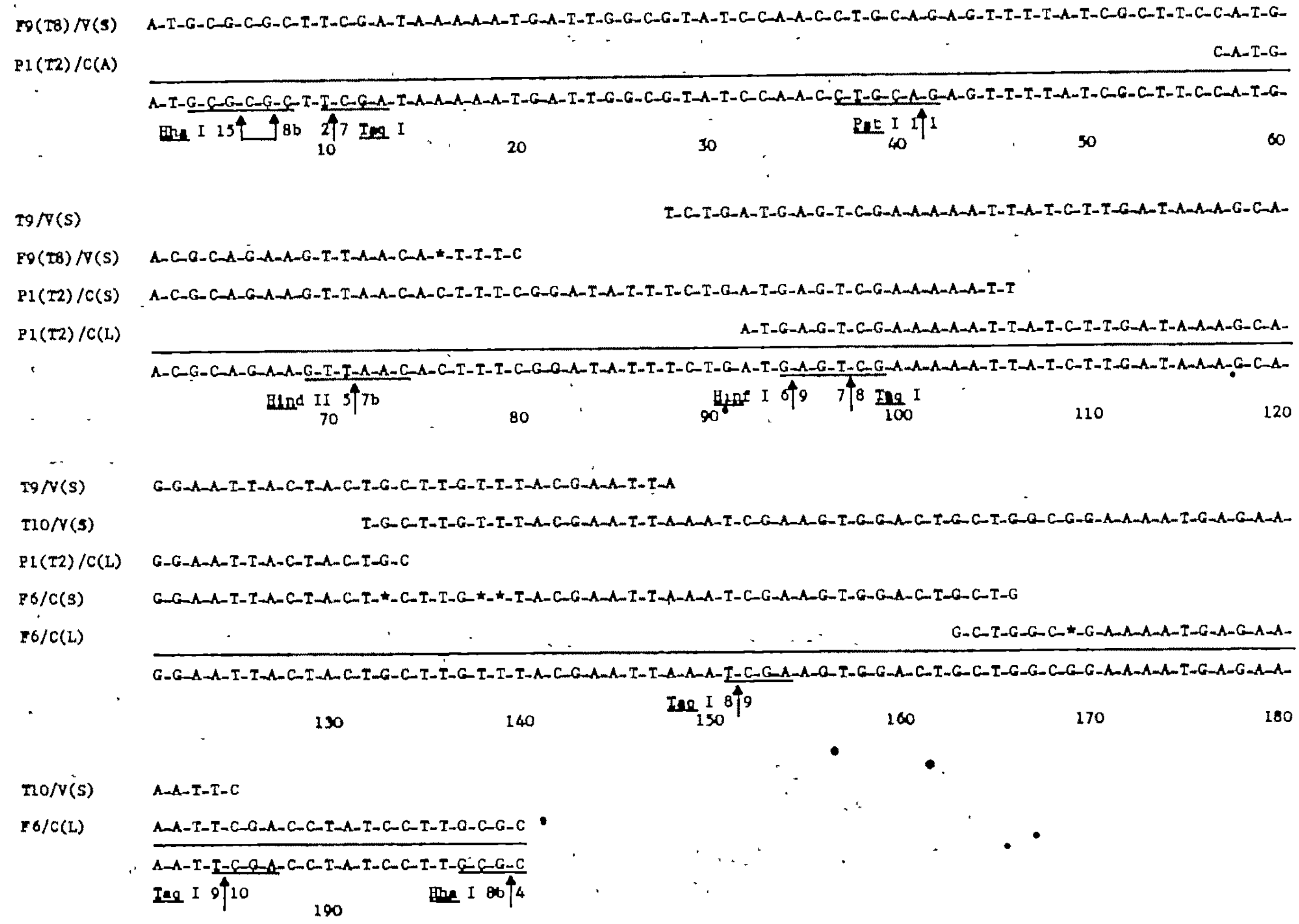
Nucleotide sequence of *A* and *B* mutants

Gene *A* mutants (*am*18, *am*35) have been mapped[8] between the left end of *Hha*I fragment 8b (fragment 7b in ref. 8) and the right end of *Hin*dII fragment 5. The mutant *am*18 and its revertant *ts*116 do not recombine to product *wt* progeny at a frequency above background. These experiments set an upper limit of approximately three nucleotides on the distance separating these mutants[9]. The *B* gene mutant (*ts*116) maps close to, or in, the *Hin*dII 5/7b site[10]. Consequently, the mutant sequences can be determined very easily using the plus and minus method by priming from the *Hin*fI 6/9 site on the mutant viral strand template. The results of these determinations for *am*3 (*E* gene mutant; wild type in this region), *am*18, *am*35 and *ts*116 are shown in Fig. 3. The independently isolated *A* gene mutants, *am*18 and *am*35, show the same altered nucleotide sequence. In the viral strand, this is a C → T transition at nucleotide 64 (Fig. 2) which results in a CAG → TAG change producing an amber codon in the reading frame which has been assigned to gene *A* (N.L.B. and M.S., in preparation). No other alterations in the *am*18 or *am*35 sequences can be seen between the *Hin*dII 5/7b site and the *Pst*I site (Fig. 2). This transition also results in an amino acid change (Ala→Val) in the reading frame assigned to gene *B* on the basis of the phasing of the ribosome binding site[3,4] and the gene *B* mutant, *am*16. This assignment of the *am*18, *am*35 (gene *A*) mutation to a specific site to the right of the *am*16 (gene *B*) as originally suggested by recombination mapping[1,9,11], and to a different reading frame, verifies the overlap of genes *A* and *B*.

The gene *B* mutant, *ts*116, was isolated as a revertant of *am*18. The mutation is a G → C change at nucleotide 66, which produces a TAG→TAC (amber→Tyr) codon change in the gene *A* reading frame. In gene *B*, the mutation results in a GAA→CAA (Glu→Gln) codon change. The resultant change in charge may be responsible for the *ts* phenotype.

The original stocks of these *am* mutants were obtained after nitrous acid treatment of viral DNA. The mutations characterised so far include G → A (*am*3,34; *am*N11,27) (ref. 2), C → T (*am*18,35) and G → T (*am*16). This variety of mutations illustrates the caution which should be exercised in predicting the consequences of chemical mutagenesis[12].

Fig. 2 Summary of the plus and minus sequence determination of the *Hha*I fragment 8b region. At the left of each determination is indicated the fragment used as the origin of the plus and minus priming. (The single letter designations used for restriction endonucleases are: *Taq* I (T), *Hin*fI, (F); and *Pst*I, (P)) Fragments indicated in parentheses are the primers used when these differed from the one corresponding to the origin (for example, F9(T8) indicates that T8 was the primer with the origin at the adjacent *Hin*fI site). The letters V and C indicate that the template was viral or complementary strand DNA, respectively. The designations (S) or (L) indicate the use of a short (4 h) or long (8 h) gel electrophoresis run.



```
F9(T8)/V(S)   A-T-G-C-G-C-G-C-T-T-C-G-A-T-A-A-A-A-A-T-G-A-T-T-G-G-C-G-T-A-T-C-C-A-A-C-C-T-G-C-A-G-A-G-T-T-T-T-A-T-C-G-C-T-T-C-C-A-T-G-
P1(T2)/C(A)                                                                                                                   C-A-T-G-
              ------------------------------------------------------------------------------------------------------------------------
              A-T-G-C-G-C-G-C-T-T-C-G-A-T-A-A-A-A-A-T-G-A-T-T-G-G-C-G-T-A-T-C-C-A-A-C-C-T-G-C-A-G-A-G-T-T-T-T-A-T-C-G-C-T-T-C-C-A-T-G-
               Hha I 15↑  ↑8b 2↑7 Taq I                                        Pst I 1↑1
                         10           20                  30                  40                50                  60

T9/V(S)                                                             T-C-T-G-A-T-G-A-G-T-C-G-A-A-A-A-A-T-T-A-T-C-T-T-G-A-T-A-A-A-G-C-A-
F9(T8)/V(S)   A-C-G-C-A-G-A-A-G-T-T-A-A-C-A-*-T-T-T-C
P1(T2)/C(S)   A-C-G-C-A-G-A-A-G-T-T-A-A-C-A-C-T-T-T-C-G-G-A-T-A-T-T-T-C-T-G-A-T-G-A-G-T-C-G-A-A-A-A-A-T-T
P1(T2)/C(L)                                                                 A-T-G-A-G-T-C-G-A-A-A-A-A-T-T-A-T-C-T-T-G-A-T-A-A-A-G-C-A-
              ------------------------------------------------------------------------------------------------------------------------
              A-C-G-C-A-G-A-A-G-T-T-A-A-C-A-C-T-T-T-C-G-G-A-T-A-T-T-T-C-T-G-A-T-G-A-G-T-C-G-A-A-A-A-A-T-T-A-T-C-T-T-G-A-T-A-A-A-G-C-A-
                        Hind II 5↑7b                                Hinf I 6↑9  7↑8 Taq I
                         70               80                  90                100                 110                 120

T9/V(S)       G-G-A-A-T-T-A-C-T-A-C-T-G-C-T-T-G-T-T-T-A-C-G-A-A-T-T-A
T10/V(S)                            T-G-C-T-T-G-T-T-T-A-C-G-A-A-T-T-A-A-A-T-C-G-A-A-G-T-G-G-A-C-T-G-C-T-G-G-C-G-G-A-A-A-A-T-G-A-G-A-A-
P1(T2)/C(L)   G-G-A-A-T-T-A-C-T-A-C-T-G-C
F6/C(S)       G-G-A-A-T-T-A-C-T-A-C-T-*-C-T-T-G-*-*-T-A-C-G-A-A-T-T-A-A-A-T-C-G-A-A-G-T-G-G-A-C-T-G-C-T-G
F6/C(L)                                                                                           G-C-T-G-G-C-*-G-A-A-A-A-T-G-A-G-A-A-
              ------------------------------------------------------------------------------------------------------------------------
              G-G-A-A-T-T-A-C-T-A-C-T-G-C-T-T-G-T-T-T-A-C-G-A-A-T-T-A-A-A-T-C-G-A-A-G-T-G-G-A-C-T-G-C-T-G-G-C-G-G-A-A-A-A-T-G-A-G-A-A-
                                                             Taq I 8↑9
                        130               140                  150                160                 170                 180

T10/V(S)      A-A-T-T-C
F6/C(L)       A-A-T-T-C-G-A-C-C-T-A-T-C-C-T-T-G-C-G-C
              ---------------------------------------
              A-A-T-T-C-G-A-C-C-T-A-T-C-C-T-T-G-C-G-C
              Taq I 9↑10             Hha I 8b↑4
                         190
```

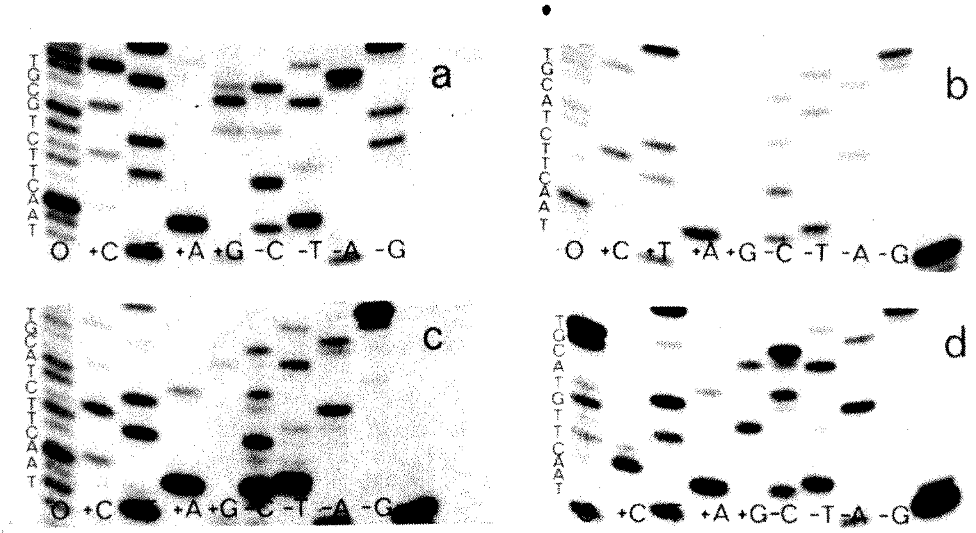


Fig. 3 Sequences of the *am*18, *am*35 and *ts*116 mutations. The *am*3 (wild type in this region) sequence is also shown. *Taq*I fragment 8 was used as primer with *Hin*fI as the datum restriction endonuclease with either (*a*) *am*3, (*b*) *am*18, (*c*) *am*35 or (*d*) *ts*116 viral DNA as template. The same portion of the autoradiogram of each plus and minus determination is shown with the sequence of the complementary strand deduced. The viral strand sequences, positions 60–74 (Fig. 2), are therefore:

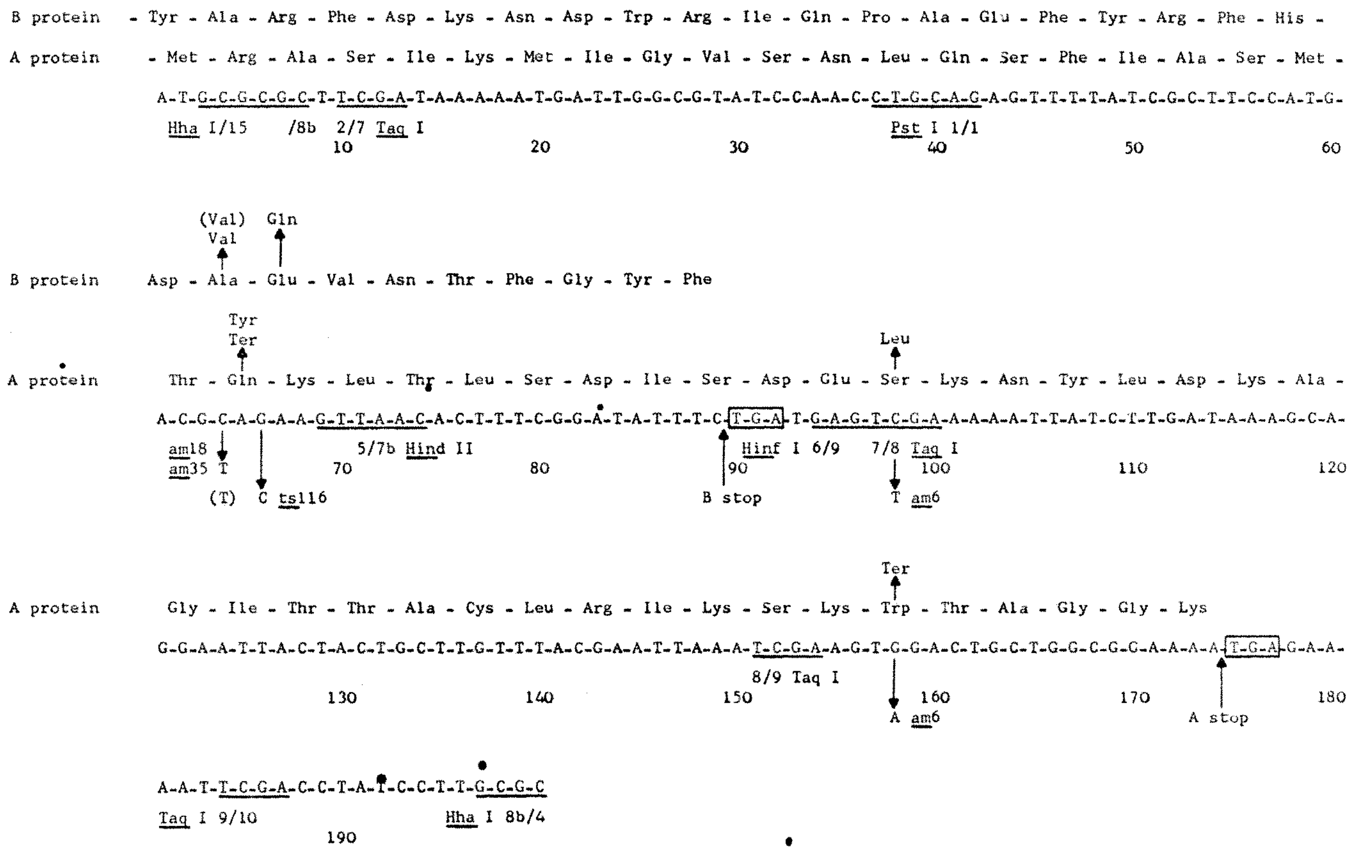
```
am3 ('wt')    G-A-C-G-C-A-G-A-A-G-T-T-A-A-C
               .   , .   , .   , .   , .

am18 (A)      G-A-C-G-T-A-G-A-A-G-T-T-A-A-C
               .   , .   , .   , .   , .

am35 (A)      G-A-C-G-T-A-G-A-A-G-T-T-A-A-C
               .   , .   , .   , .   , .

ts116 (B)     G-A-C-G-T-A-C-A-A-G-T-T-A-A-C
               .   , .   , .   , .   , .
                                     ↑
                             Hind II 5 7b
```

Dots indicate the reading frame for gene *A* and commas that for gene *B*.



```
B protein   - Tyr - Ala - Arg - Phe - Asp - Lys - Asn - Asp - Trp - Arg - Ile - Gln - Pro - Ala - Glu - Phe - Tyr - Arg - Phe - His -

A protein     - Met - Arg - Ala - Ser - Ile - Lys - Met - Ile - Gly - Val - Ser - Asn - Leu - Gln - Ser - Phe - Ile - Ala - Ser - Met -

            A-T-G-C-G-C-G-C-T-T-C-G-A-T-A-A-A-A-A-T-G-A-T-T-G-G-C-G-T-A-T-C-C-A-A-C-C-T-G-C-A-G-A-G-T-T-T-T-A-T-C-G-C-T-T-C-C-A-T-G-
            Hha I/15   /8b  2/7 Taq I                                     Pst I 1/1
                              10                  20                  30                  40                  50                  60


                (Val)  Gln
                 Val    ↑
                  ↑     |
B protein   Asp - Ala - Glu - Val - Asn - Thr - Phe - Gly - Tyr - Phe

                 Tyr
                 Ter                                                        Leu
                  ↑                                                          ↑
A protein    Thr - Gln - Lys - Leu - Thr - Leu - Ser - Asp - Ile - Ser - Asp - Glu - Ser - Lys - Asn - Tyr - Leu - Asp - Lys - Ala -

            A-C-G-C-A-G-A-A-G-T-T-A-A-C-A-C-T-T-T-C-G-G-A-T-A-T-T-T-C-T-G-A-T-G-A-G-T-C-G-A-A-A-A-A-T-T-A-T-C-T-T-G-A-T-A-A-A-G-C-A-
            am18 ↓  |       5/7b Hind II                      ↑ Hinf I 6/9   7/8 Taq I
            am35 T  |     70                 80               |90              ↓ 100                110                 120
                (T) ↓ C ts116                               B stop             T am6


                                                                            Ter
                                                                             ↑
A protein    Gly - Ile - Thr - Thr - Ala - Cys - Leu - Arg - Ile - Lys - Ser - Lys - Trp - Thr - Ala - Gly - Gly - Lys

            G-G-A-A-T-T-A-C-T-A-C-T-G-C-T-T-G-T-T-T-A-C-G-A-A-T-T-A-A-A-T-C-G-A-A-G-T-G-G-A-C-T-G-C-T-G-G-C-G-G-A-A-A-A-T-G-A-G-A-A-
                                                                     8/9 Taq I  |                             ↑
                              130                140                150         ↓ 160                170      |           180
                                                                                A am6                      A stop

            A-A-T-T-C-G-A-C-C-T-A-T-C-C-T-T-G-C-G-C
            Taq I 9/10            Hha I 8b/4
                              190
```

Fig. 4 The sequence of ΦX174 viral DNA in the region of gene *B* and the C terminus of gene *A*. The amino acid sequences of the gene *A* and gene *B* proteins predicted from the DNA sequence are shown together with the changes resulting from the mutations *am*18 and the subsequent *ts*116, *am*35 and *am*6.

The changes induced in the second gene by mutation in its overlapping companion are not necessarily restrictive, as evidenced by the Leu→Phe change in gene *A* which accompanies the *am*16 mutation in gene *B*, the Ala→Val change in gene *B* accompanying the *am*18,35 mutation in gene *A*, and the reversion of *am*18, which by two steps induces a Gln → Tyr in gene *A*. There is no evidence for any impairment in protein function due to these changes, whereas in *ts*116 the second amino acid change (Glu→Gln) in gene *B* which follows the initial change (Ala → Val) induced by the gene *A* amber mutation evinced a phenotypic response (*ts*).

C termini of A and B proteins

The sequence determination and establishment of the *A* gene reading frame taken together with the *B* gene reading frame allow the prediction of the C termini of the two proteins (Fig. 4). The B protein is 120 amino acids long with a molecular weight of 13,845. The *A* gene extends for 85 nucleotides (28 codons) beyond the C terminus of gene *B*. If, as has been suggested (N.L.B. and M.S., in preparation), the gene *A*/gene *B* overlap arose by read-through of a previously shorter gene *A*, then a question arises about the original role of the DNA beyond the C terminus of gene *B*. The mutant *am*6 is a double amber mutant mapping in fragments F9 (gene *A*) and Z7 (gene *E*) (P. Weisbeek, personal communication). In gene *E* the mutation is identical to *am*3. The gene *A* mutation has also been sequenced and is a G → A change at nucleotide 158. This gives an amber (TAG) codon, and would result in a gene *A* product six amino acids shorter than in the wild type. In addition there is a C → T change at nucleotide 98 which would result in a missense Ser → Leu in the gene *A* protein, and preliminary results suggest yet another change in this region. The resulting gene product is impaired in function, indicating that there is some requirement for the region of the A protein coded by the DNA beyond the end of gene *B*.

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Photochemical attachment of cyclic AMP binding protein(s) to the nuclear genome

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The nature of the cyclic AMP–receptor–nucleus interactions was examined by a novel combination of two photoreactions. A photosensitive derivative of cyclic AMP, N^6-butyryl cyclic AMP, was covalently attached to its cytoplasmic receptod by photo-affinity labelling and this receptor complex was photo-crosslinked by the DNA in the rat liver nuclei. The photolytic reactions seemed to be specific since stable links were formed only when substantial noncovalent binding occurred.

CLOSE similarity between two modes of signalling to the nucleus in mammalian cell regulation, one by steroid hormones[1–5] and the other by cyclic nucleotides[6–10], has been suggested. Both steroid hormones and cyclic AMP combine specifically with a cytoplasmic receptor protein, and then the resulting ligand–receptor complex enters into the nucleus, where it binds to the genome. Little is known of the nature of these hormone–receptor–genome interactions. A requirement for the solution of this problem is the identification of the residues involved in the protein–DNA interactions in the nucleus.

I intend to study the chemistry of the ligand–receptor and receptor–genome interactions and to freeze the *in situ* interacting residues by the covalent attachment of the ligand to the receptor and the receptor complex to the genome in the intact nucleus, so that these residues can be identified. I report here the combined use of two well-known photolabelling techniques. In one, photo-affinity labelling[11–13], the ligand derivative is covalently attached to the receptor protein to identify the ligand binding site. In the other, the photo-induced crosslinking reaction[11,14–18], the protein and nucleic acid are linked together to locate the segments of the macromolecules that are in close contact. This method of 'photo-affinity crosslinking' is based on the ability of the mobile cytoplasmic receptor to bind its ligand specifically and then to migrate to the nuclear site, thus delivering a photosensitive probe to form a ternary nuclear complex.

Photo-affinity crosslinking was used to study the covalent attachment of cyclic AMP to its 'receptor' and the crosslinking of the receptor complex to DNA in the intact nucleus. (In this paper the term 'receptor' is used to signify a high affinity cyclic AMP binding site.) The previously described cyclic-AMP–receptor–nucleus *in vitro* system from rat liver was used[19]. The N^6-butyryl derivative of cyclic AMP (NBcAMP)[20–21] was chosen for photo-affinity labelling because the carbonyl group on the acyl side chain can be electronically excited by irradiation to a chemically reactive species[22]. This excitation can produce a variety of free radicals from the rupture of the bond in the α position to the carbonyl (α cleavage) or a 1,5 hydrogen shift of the γ-hydrogen (hydrogen abstraction) and leads to the covalent bond formation between the ligand and the receptor[11,22].

Preliminary photo-affinity labelling studies of the cytoplasmic receptor with NBcAMP encouraged us to explore the applicability of receptor site labelling in a more complex system, the cell nucleus. It was expected that NBcAMP would bind specifically to its cytoplasmic receptor proteins,

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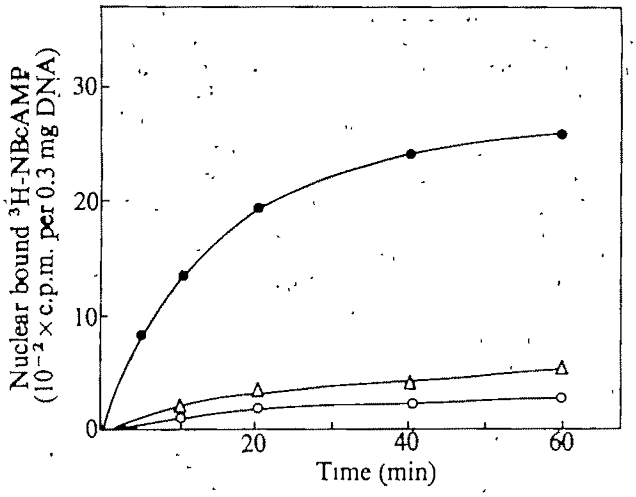


Fig. 1 Time course, temperature dependence and cytosol requirement for the specific uptake of ^{3}H-NBcAMP by isolated rat liver nuclei. Purified liver nuclei were prepared as before[19]. Nuclei (0.30 mg of DNA) were suspended in 0.5 ml of buffer A (0.30 M sucrose, 25 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 3 mM mercaptoethanol, 50 mM Tris-HCl, *p*H 7.5) and incubated at 4 °C or 25 °C for the indicated times with 0.35 ml of liver cytosol[10] (~15 mg of protein, ml^{-1}, 105,000*g* supernatant fraction and equilibrated with buffer B (25 mM KCl, 3 mM mercaptoethanol, 1 mM EDTA, 15% glycerol, 50 mM Tris-HCl, *p*H 7.5) either by gel filtration through Sephadex G-25 or by dialysis) in the presence of 1×10^{-7} M ^{3}H-NBcAMP (New England Nuclear) Following incubation, the mixture was chilled, diluted with 2.5 ml cold buffer A, centrifuged (900*g*. 8 min) and the pellet washed three times with 4 ml of buffer A and finally the nuclear pellet was solubilised (in 0.5 ml of 0.20 M NaOH containing 0.025% SDS) Aliquots were assayed for radioactivity (by liquid scintillation counter) and for DNA (by A_{260}) Each value represents the average of 6 determinations The specific binding of ^{3}H-NBcAMP was calculated from the difference in the assay performed in the presence or absence of 500-fold excess of nonradioactive NBcAMP. ●, Incubation at 25 °C with cytosol; △, 25 °C, no cytosol; ○, 4 °C with cytosol.

that this complex, on activation, should migrate into the nucleus to form a nuclear complex, and that photolysis of this nuclear complex should ultimately result in covalent attachment of cyclic AMP derivatives to its receptor and in photochemical crosslinking of the receptor to nuclear DNA. The validity of these predictions was tested.

Nuclear binding of NBcAMP–receptor complex

There is a cytosol requirement for the *in vitro* transfer of cyclic AMP from cytoplasm to nucleus[19]. Here I demonstrate, in a similar system, the reversible binding of the NBcAMP–receptor complex to the purified nuclei. When rat liver nuclei were incubated at 25 °C with ^{3}H NBcAMP in the presence of cytosol, there was time dependent binding of the radioactivity to the nucleus (Fig 1). In the absence of cytosol, ^{3}H-NBcAMP bound poorly to the nuclei Neither heat denatured cytosol, bovine serum albumin (BSA) alone, nor trypsin treated cytosol could stimulate the transfer of ^{3}H-NBcAMP (Table 1), suggesting that the entire cytoplasmic receptor complex, and not the cyclic nucleotide alone, was transferred to the nucleus

I have shown that nuclear binding of cyclic AMP, in close similarity to the steroid systems, requires a time, temperature and ligand dependent transformation of the receptor complex, resulting in its increased affinity for the chromatin or for DNA. This was termed the 'activation' step. A similar time and temperature dependence was demonstrated for NBcAMP–receptor complex (Fig. 1). At 25 °C, the transfer was nearly complete in 30 min, but at 4 °C the reaction was much slower.

The binding of ^{3}H-NBcAMP to cytosol receptor seems to be specific since the interaction could be prevented only by the addition of a large excess (400-fold) of nonradioactive cyclic AMP or NBcAMP, but not by excess of ATP, AMP, or GMP (Table 1) The fact that the NBcAMP binding was inhibited by cyclic AMP as well as by NBcAMP suggests that they both interacted with the same receptor site[19,21].

The nuclear binding of the receptor complex seems to be noncovalent, since the NBcAMP–receptor complex is extractable from the nucleus with 0.4 M KCl In addition, there is no apparent chemical change in the cyclic nucleotide during the nuclear binding, since at least 90% of the bound radioactivity was shown to be identical with ^{3}H-NBcAMP on TLC[19,22].

Photolytic incorporation of NBcAMP–complex into the nucleus

Preliminary photo-affinity labelling studies of the cytoplasmic receptor with ^{3}H-NBcAMP (in preparation) en-

Table 1 Cytosol requirement and comparison of non-covalent and covalent nuclear binding of ^{3}H-NBcAMP

Comparison of non-covalent and covalent binding	Non-covalent nuclear binding (c.p.m. per 0.7 mg of DNA)	TCA precipitated counts after 15 min ultraviolet* (c.p.m.)
Cytosol	5,360	2,170 (140 no UV)
No cytosol	1,040	260
Heat denatured cytosol (15 min at 65 °C)	1,270	310
Trypsin treated cytosol†	1,210	320
Cytosol replaced by bovine serum albumin (12 mg ml^{-1} in buffer B)	1,330	370
Cytosol+nonradioactive cyclic AMP (5×10^{-5} M)	320	140
Cytosol+nonradioactive NBcAMP (5×10^{-5} M)	410	180
Cytosol+AMP (5×10^{-5} M)	5,210	2,230
Cytosol+ATP (5×10^{-5} M)	5,460	2,290
Cytosol+GMP (5×10^{-5} M)	5,370	2,240
Enzymatic digestions of the irradiated ^{3}H-NBcAMP ternary nuclear complex‡		
DNase	—	2,180
RNase	—	2,220
Trypsin	—	260

Nuclei (0.7 mg of DNA) and cytosol (0.75 ml; 12–15 mg protein per ml) were incubated with 1×10^{-7} M ^{3}H-NBcAMP for 50 min at 25 °C. After the samples were chilled, centrifuged and washed, the nuclear bound ^{3}H-NBcAMP was determined as described in Fig. 1.

*Photolysis of the ^{3}H-NBcAMP nuclear complex (prepared as above) was carried out as described in Fig. 2 for 15 min; aliquots were assayed by TCA precipitable radioactivity.

†Cytosol was treated with trypsin (0.2 mg ml^{-1}, Worthington) for 30 min at 25 °C and then soybean trypsin inhibitor (0.35 mg ml^{-1}) was added for 5 min before incubation with the nuclei.

‡Photolysis of ^{3}H-NBcAMP ternary nuclear complex was carried out as described in Fig. 2*b*, followed by enzymatic digestion with DNase (0.1 mg ml^{-1}), RNase (0.1 mg ml^{-1}) and trypsin for 45 min at 30 °C as described in Fig 3 and finally analysed for TCA insoluble radioactivity as above.

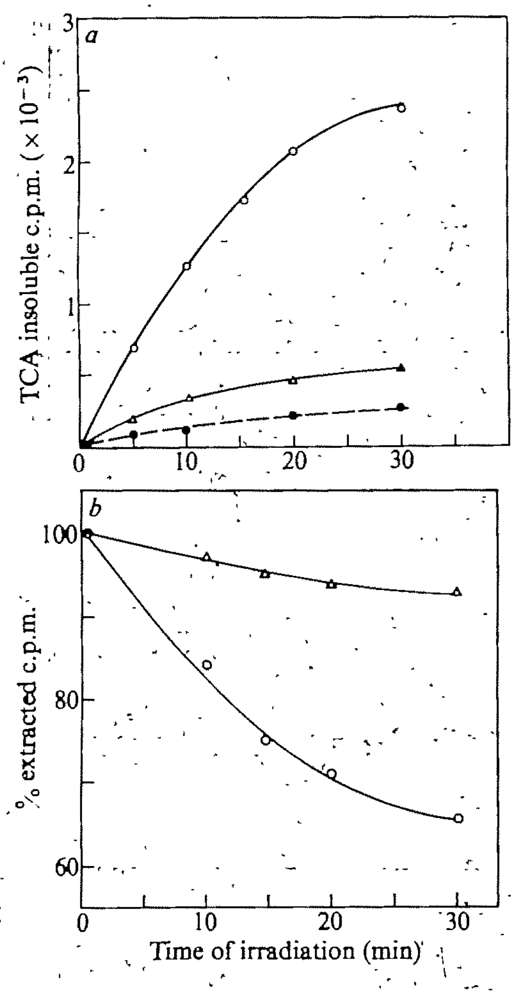


Fig. 2 Time dependence of the photolytic incorporation of ^{3}H-NBcAMP into the nuclear complex. *a*, TCA precipitable counts; the ^{3}H-NBcAMP nuclear complex was prepared, as described in Fig. 1, by incubating nuclei (1 mg of DNA) and cytosol (1.2 ml, 12–15 mg of protein ml^{-1}) at 25 °C for 50 min with ^{3}H-NBcAMP (1×10^{-7} M) and resuspended in 2.5 ml of buffer C (0.20 M sucrose, 25 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 7.5 mM mercaptoethanol, 1 mM EDTA, 20% glycerol, 50 mM Tris-HCl, *p*H 7.5) and placed into wells of a plastic culture plate on ice, equipped with magnetic stirrer. The reaction mixture was irradiated at a distance about 8 cm with a 25-W mercury lamp (General Electric, predominantly 253.7 nm radiation). At the indicated time, 0.60-ml aliquots of the irradiated mixture were assayed by TCA precipitable radioactivity (2 ml of cold 20% TCA was added to the aliquots, centrifuged, and washed 3 times with 4 ml of 5% TCA), the pellet was dissolved in 0.5 ml of 0.20 M NaOH containing 0.025% SDS and assayed for radioactivity. ^{3}H-NBcAMP–cytoplasmic receptor complex was prepared by incubating cytosol (3 ml) with ^{3}H-NBcAMP (1×10^{-7} M) for 30 min at 4 °C and equilibrated with buffer B either gel filtration (through Sephadex G-25) or by dialysis, and finally was irradiated as above. Controls were treated identically, but were not exposed to ultraviolet light. Complete nuclear incubation mixture irradiated (○); complete nuclear mixture unirradiated (control) (●); irradiated in the absence of cytosol (Δ). *b*, Percentage of radioactivity released by 0.4 M KCl versus time of irradiation; photolysis of ^{3}H-NBcAMP nuclear complex was performed in the absence and presence of 0.4 M KCl in buffer C as described above. At indicated times aliquots were removed, KCl concentration uniformly adjusted to 0.4 M, centrifuged, extracted again with 1 ml of 0.4 M KCl (in 50 mM Tris, *p*H 7.5), and the combined supernatant (nuclear extract) was assayed for the protein bound radioactivity by gel filtration (13 × 1.25-cm column of Sephadex G-25); 65–75% of the extracted nuclear radioactivity was present in the macromolecular fraction. The amount of released radioactivity from the nuclei is expressed as a percentage of the nonirradiated control. Irradiation in the absence (○) and in the presence (Δ) of 0.4 M KCl.

Table 2 Activation and photochemical fixation

	Salt extracted (c.p.m.) from nuclei (% of control)
Activation at 25 °C (control; no ultraviolet)	100
Activation followed by ultraviolet*	69
Ultraviolet exposure followed by activation†	93
Activation followed by ultraviolet and then the DNase digestion‡	98

Nuclear suspension (0.35 mg DNA) and cytosol (in the same ratio as in Fig. 1*a*) were incubated in buffer C with 1×10^{-7} M ^{3}H-NBcAMP for 50 min at 25 °C, then incubated again for 20 min at 4 °C (in the irradiation plate, as in Fig. 2, but not exposed to ultraviolet). The nuclear suspension was then extracted with 1 ml of 0.4 M KCl in 50 mM Tris, *p*H 7.5. After standing for 30 min with occasional agitation, the nuclei were pelleted and the KCl extract containing the ^{3}H-NBcAMP protein complex was analysed by Sephadex G-25 gel filtration for macromolecular bound radioactivity as described in Fig. 2*c* legend.

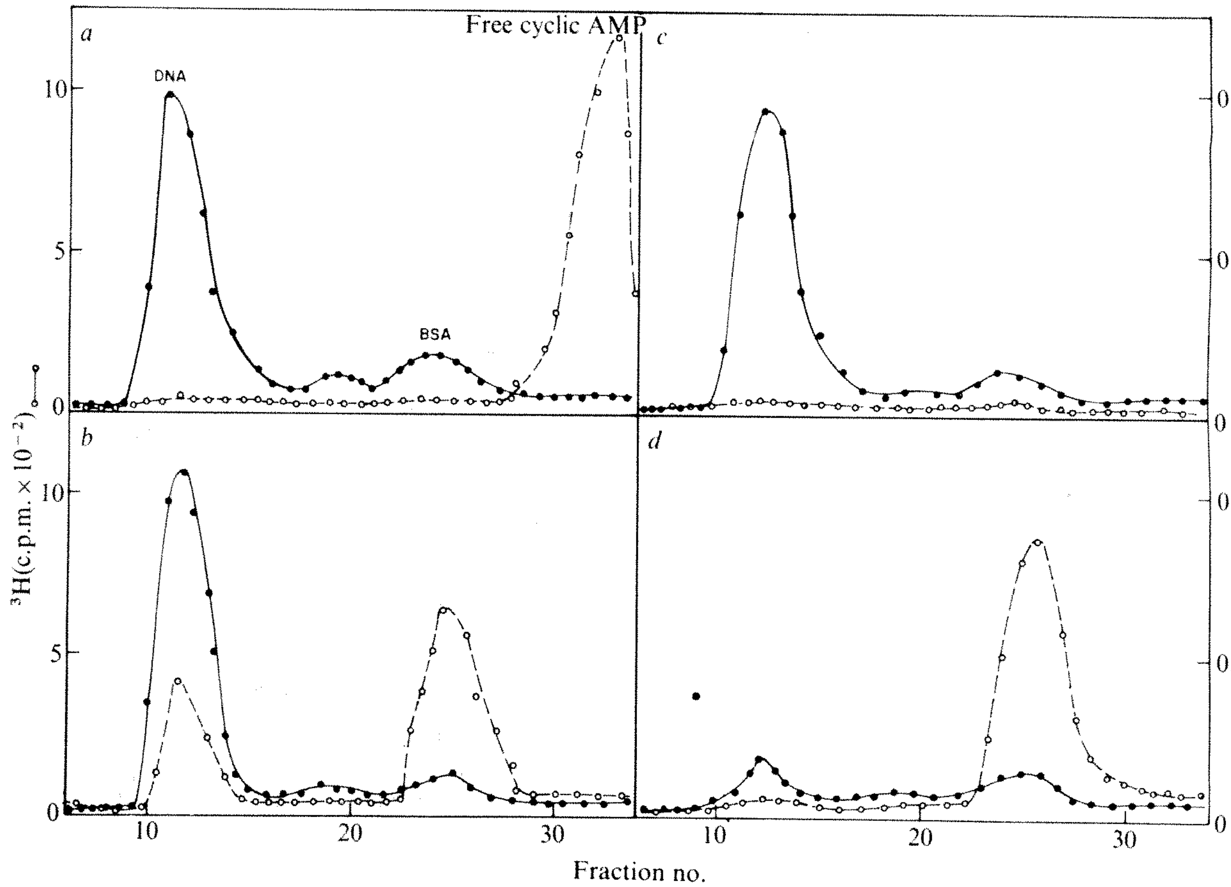
*Nuclei and cytosol were incubated as above and the nuclear suspension was irradiated for 20 min at 4 °C, extracted and macromolecular radioactivity was estimated as above. The amount of released radioactivity from the nuclei is expressed as a % of the nonirradiated control.

†Nuclei and cytosol were incubated with ^{3}H-NBcAMP in buffer C as above, but at 4 °C for 15 min and then irradiated for 20 min at 4 °C. The irradiated suspension was then activated by incubation for 50 min at 25 °C and then extracted and assayed.

‡Nuclei and cytosol were incubated with ^{3}H-NBcAMP for 50 min at 25 °C, then photolysed for 20 min. After irradiation the samples were treated with pancreatic DNase 1 (Worthington, final concentration 0.1 mg ml^{-1} in 50 mM Tris, *p*H 7.5, containing 2 mM $CaCl_2$ and 2 mM $MgCl_2$, incubated for 35 min at 30 °C with occasional shaking and terminated by addition of EDTA, to final concentration of 15 mM). For the DNase control, EDTA was added before the DNase. Extracted radioactivity was then estimated as before (mean of four experiments). The amount of released macromolecular radioactivity in the control was 2120 c.p.m. per 0.35 mg of DNA.

couraged me to explore the receptor labelling in the cell nucleus. Photolysis of the NBcAMP nuclear complex at 254 nm was followed by TCA precipitable counts as a function of time of irradiation. Results of a typical nuclear irradiation experiment (Fig. 2*a*) show that the amount of radioactivity associated with the macromolecules was directly proportional to the time of irradiation. Also, in the absence of irradiation (control), no significant radioactivity was rendered TCA precipitable. The reaction seems to be covalent, as the radioactivity remains associated with the macromolecular fraction during treatments which remove noncovalently bound ligands, such as extensive dialysis against 7 M urea, Sephadex G-25 filtration in the presence of 0.5% SDS, or 10 min boiling with TCA. The photolytic incorporation of ^{3}H into the nuclear macromolecular component seems to be specific, because the reaction could only be prevented by addition of a large excess of non-radioactive cyclic AMP or NBcAMP (Table 1), with no significant competition with other nucleotides such as AMP, ATP and GTP. Further, the photolytic process could not be stimulated in the absence of cytosol, or with cytosol replaced by heat denatured or trypsin treated cytosol, or serum proteins (Table 1*b*). Thus an intact cytoplasmic protein seems to be required for the photochemical reaction, as well as for the non-covalent nuclear binding of NBcAMP.

When the ^{3}H-NBcAMP nuclear complex was incubated in the dark and then subjected to extraction in 0.4 M KCl, most of the radioactive receptor complex was recovered in the extract. When the same complex was irradiated, there was a time dependent decrease in the recovery of the complex this decrease was not seen if the photolysis itself was carried out in the presence of 0.4 M KCl (Fig. 2*b*). About 25% of the radioactive complex can no longer be extracted with salt (as compared with the non-irradiated control), suggesting that a portion of the complex becomes



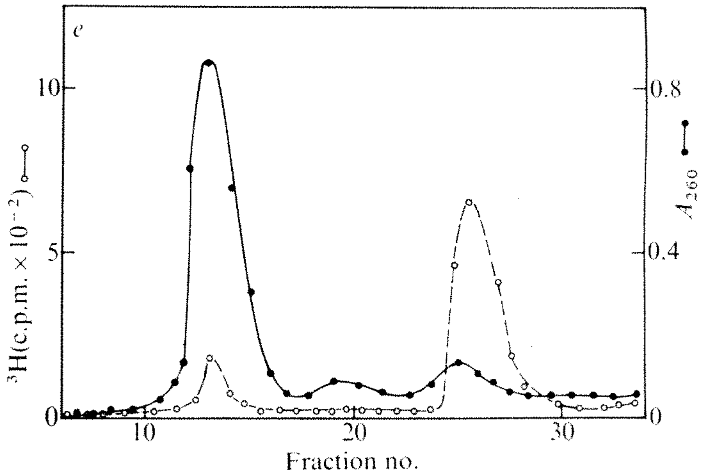


Fig. 3 Characterisation of the irradiated ^{3}H-NBcAMP nuclear complex by SDS Sepharose 2B chromatography. DNA (calf thymus), protein (BSA) and ^{3}H-NBcAMP were used as markers and their respective positions are indicated. Photolysis (for 15 min) of ^{3}H-NBcAMP nuclear complex (1 mg DNA) was carried out as for Fig. 2. In some experiments the noncovalently bound ^{3}H-NBcAMP was removed by TCA precipitation before solubilisation. The TCA or nuclear pellets were then solubilised in about 1 ml of 1% SDS (containing 0.025 M EDTA, 0.05 M metacaptoethanol, 0.1 M Tris-HCl *p*H 8) by heating it at 60 °C for 20 min, with occasional shaking and the sample was applied to a Sepharose 2B column (1.3 × 45 cm) equilibrated (at room temperature) with Tris-SDS buffer (0.15 M KCl, 0.01 M EDTA, 0.05 M mercaptoethanol, 0.1 M Tris-HCl *p*H 8, 0.5% SDS) and 1-ml fractions were collected for determination of absorbancy and radioactivity. After irradiation and before solubilisation, all samples were sonicated (to a DNA molecular weight about 5×10^6). *a*, Elution profile of non-irradiated control was treated identically but not exposed to ultraviolet and to TCA. *b*, Profile of the photolysis sample (with TCA precipitation). *c*, Chromatography of the irradiated complex after trypsin digestion. Following the irradiation, samples were subjected to trypsin treatment (Worthington, final concentration 0.1 mg ml^{-1}, incubated at 30 °C for 45 min, and precipitated with 50% of TCA to final concentration of 10%). The precipitates were collected by centrifugation and washed (sequentially with acetone 3 ml and ether 2 ml) and the pellets were finally suspended in 1% SDS, and analysed by SDS Sepharose chromatography as above. *d*, Chromatography of the irradiated complex after DNase digestion. After photolysis the samples were digested with pancreatic DNase 1 (Worthington, final concentration 0.1 mg ml^{-1} in 50 mM Tris-HCl buffer, *p*H 7.5, containing 2 mM $CaCl_2$ and 2 mM $MgCl_2$ incubated for 40 min at 30 °C with occasional shaking and terminated by TCA precipitation). The digested samples were analysed by SDS Sepharose 2B chromatography. *e*, Irradiation in presence of 0.4 M KCl; ^{3}H-NBcAMP nuclear complex was suspended in 1 ml of buffer C containing 0.4 M KCl irradiated for 15 min as before and analysed by SDS Sepharose 2B chromatography.

'fixed' in the nucleus after 20 min of irradiation. Alternatively, the receptor site may be destroyed during the irradiation, with the radioactive ligand being somehow retained by the nucleus.

If the ^{3}H receptor complex is fixed in the nucleus by covalent attachment, then the nature of the linkage could be characterised by enzymatic digestion. Trypsin and pronase digestion of the photolysed nuclear complex rendered most of the radioactivity TCA soluble (Table 1), but treatment with DNase or RNase did not. DNase treatment, however, resulted in drastic liberation of the fixed ^{3}H-receptor complex from the nuclei, so that the previously fixed radioactivity became extractable with 0.4 M KCl (Table 2). Digestion with DNase suggests that DNA is involved in the nuclear fixation and that the receptor site was probably not destroyed during the irradiation. These experiments are consistent with (but do not prove) the hypothesis that during photolysis the ligand becomes covalently attached to the cytoplasmic receptor and the receptor complex, in turn, is crosslinked to the nuclear DNA.

Specificity of the photochemical reaction

Is the photochemical reaction specific, or is it simply a random collision of free radicals and/or other active species generated during the irradiation process? If the photo-affinity labelling is specific, the reagent molecule would probably bind reversibly and specifically to the receptor site(s), and then bind covalently after photolysis. Thus, conditions which would interfere with specific reversible binding would also prevent covalent binding. I expected first, that a denatured or degraded receptor which is unable to interact either with ligand or with the nuclei would be unable to mediate the photochemical attachment of ligand to receptor or receptor to nuclei. Second, dissociating conditions in 0.4 M KCl which prevent the reversible nuclear binding of the receptor complex should also prevent the photochemical attachment to the nuclei. Third, absence of the heat activation step should have a similar effect. Thus, if the photochemical reaction is specific, then in the absence of reversible binding, there should be no covalent bound formation at the nuclear site.

The results support these predictions. First, degraded receptor did not promote the covalent photoreactions, or the noncovalent nuclear binding of the receptor complex (Table 1). Second, in the presence of 0.4 M KCl when the receptor complex was not associated with the nucleus (Fig. 2*b*), photolysis yielded only affinity labelling at the receptor site, but the receptor was not attached to the nucleus. Third, to verify the heat activation requirement, experiments were carried out in the reverse sequence when the activation (binding) step was preceded by irradiation, again no nuclear linked receptor was observed (Table 2). These results are consistent with the implication that the photochemical reactions are specific: stable links are formed only when substantial noncovalent binding occurs.

Characterisation of nuclear photolysis products

The chemical characterisation of the product formed between ^{3}H-NBcAMP, protein and DNA during irradiation of the nucleus was performed using enzymatic digestions, followed by Sepharose 2B chromatography in the presence of detergent (0.5 SDS) to denature and to dissociate all noncovalently linked molecules. A typical chromatogram of nonirradiated nuclei is shown in Fig. 3*a*. The DNA appeared in the void volume, followed by proteins, and finally by free radioactive ligand. In the presence of the detergent, there was essentially no radioactivity associated with either the DNA or the protein fractions. After 15 min of irradiation, however, there was a striking change in the chromatographic profile (Fig. 3*b*). Two new macromolecular radioactive peaks appeared: the first, in the void volume, coeluted with DNA, and the second, larger peak, appeared with proteins. For the first peak, I have considered the following possible linkages formed during the irradiation between ^{3}H-NBcAMP (cA) and macromolecules: cA–DNA, cA–DNA–protein, DNA–cA–protein, cA–protein–protein(s). These structural alternatives might be distinguished by specific enzymatic digestions. After DNase digestion (Fig. 3*d*), no radioactivity remained associated with the DNA peak, no additional free radioactive ligand was liberated, and all radioactivity migrated with the protein fraction. In contrast, trypsin treatment (Fig. 3*c*) liberated all macromolecular bound radioactivity. Thus, there is probably a protein 'bridge' to link the ^{3}H ligand to DNA, for example ^{3}H–cA–protein–DNA, and there seems to be no direct association between the ligand and DNA.

I have indicated that the dissociating condition prevented both the noncovalent nuclear binding and the covalent attachment of receptor to the nuclei. To substantiate this conclusion, I analysed the chromatographic profile of the reaction products. When the ^{3}H-receptor complex and nucleus were irradiated under dissociating conditions (0.4 M KCl), (Fig. 3*e*), radioactivity was associated mainly with the protein fraction, and only minor amounts with the DNA. Furthermore, when the photolysis was carried out before the activation step, there was essentially no radioactivity in the DNA fraction (data not shown). These experiments further demonstrate that covalent attachment is observed only where substantial noncovalent association occurs.

The alternative that the covalent reaction occurs in the nuclei between the NBcAMP and certain proteins, but without the involvement of the cytoplasmic receptors, is unlikely because essentially no covalent photo-reaction occurs in the absence of the cytosol. It remains possible that the ligand is somehow transferred from its original receptor to another nuclear constituent ('pseudo-photo-affinity labelling', ref. 24). If the nuclear crosslinking product did not involve DNA, this would be difficult to reconcile with the DNase sensitivity of the photo-adduct. Finally, the possibility that a crosslinking reaction occurs with a relatively unspecific part of nuclei, such as nonchromatin material, is also unlikely, as, on photolysis, the same type of crosslinking product was obtained when the nuclei was replaced by purified chromatin (Kallos, unpublished), but not with DNase treated nuclei.

Conclusion

The formation of a stable ternary complex, presumably of covalent nature, has been achieved through specific photolysis of the NBcAMP–protein–nuclei system. The exact chemical nature of the stable joint nuclear complex is not yet known. It probably involves the covalent attachment of NBcAMP to the receptor site and the crosslinking formation between reactive DNA base(s) and protein residues that are in close proximity in the ternary complex.

These results suggest that the optimal noncovalent, as well as covalent, nuclear binding of cyclic AMP require the presence of an intact cytoplasmic receptor. I propose the following reaction sequences (scheme 1) for the reversible interactions (sequence a): (1) the cyclic AMP derivative, NBcAMP (cA), binds specifically and reversibly to a soluble cytoplasmic receptor protein (giving cA·R); (2) this is followed by a temperature-dependent (T°) activation step[19] (cA·R′); and (3) there is an association of this activated complex with the nucleus (cA·R′.N). The photolysis of this reversible nuclear complex (step b) results in the covalent link formation (cA*–R*–N*). The asterisks represent the as yet unidentified photochemically altered components. When the receptor complex is not associated with the nucleus; for example, in the presence of 0.4 M KCl or in the absence of activation, photolysis yields only an affinity label at the cyclic AMP site (cA*–R*), but the receptor is not attached to the nucleus.

The function of the receptor protein, possibly the regulatory subunit of a protein kinase, remains to be determined. It is interesting to note that cyclic AMP dependent protein kinases, which can bind to nuclei (or to DNA), have recently been described in the thymus[25] and in the ovary[26]. Although the photochemical reaction was shown to be specific by several criteria, one must be cautious in the interpretation of the results due to the tendency of the receptors to aggregate[5,27,28].

The particular value of photo-affinity crosslinking is to provide a chemical tool for structural study of the locus of the genome which interacts with the receptor. The present study can only be considered preliminary until the photoproducts are separated and further identified.

This work is dedicated to the late Gordon M. Tomkins (1926–1975), my colleague for 11 years. I thank B. Alberts, V. C. Allfrey, C. B. Anfinsen, R. Axel, C. Cantor, B. S. Cooperman, J. S. Edelman, T. M. Fasy, M. A. Lee, E. Zeff and N. Farber for discussions, and Frances Flug for technical assistance. The work was carried out in Dr Lee's laboratory supported by American Cancer Institute and U.S. Public Health Service grants.

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letters to nature

Cosmogony and the magnitude of the dimensionless gravitational coupling constant

SIMPLE arguments involving gravitational fragmentation indicate that for the Universe to contain galaxies and stable nuclear-burning stars, the dimensionless gravitational coupling constant $\alpha_g (= Gm_p^2/\hbar c)$ must lie within several orders of magnitude of its actual value (5×10^{-39}). The characteristic mass of a galaxy is shown to be $\alpha^5\alpha_g^{-2}$ nucleons. If galaxies and galaxy clusters form by gravitational instability and fragmentation of inhomogeneities in the early Universe, the large-scale structure of the Universe imposes a lower bound, and the instability requirement an upper bound (equal to $\alpha_g^{-1/4}$), on the entropy per particle.

Gravity is an exceedingly weak force. A convenient measure of its magnitude is given by the dimensionless constant $\alpha_g = Gm_p^2/\hbar c$ (the ratio of gravitational to electromagnetic forces between a pair of protons multiplied by the atomic fine structure constant $\alpha (= e^2/\hbar c)$, and equal to 5×10^{-39}). The origin and significance of this number have provided a continuing source of speculation among physicists, whose very existence (and indeed the existence of all life) may be contingent on its value[1,2].

Certain elementary and speculative considerations show that for the Universe to contain galaxies and nuclear-burning stars, α_g must lie within a few orders of magnitude of its actual value. In outlining these arguments, I shall also show how the magnitude of one of the most fundamental of cosmological constants, namely the entropy per baryon, can be related to the scale of the large-scale structure of the Universe and the value of α_g. Two significant large number coincidences involving α_g that I will not discuss are the total number of baryons in the observable Universe ($N_u \sim \alpha_g^{-2}$) and the age of the Universe ($\sim \alpha_g^{-1}\hbar/m_pc^2$) (see refs 1–5).

My discussion is based on some recent work[8] on the fragmentation of self-gravitating gas clouds. The concept of gravitational instability was originally presented as a quantitative criterion by Jeans, but Hoyle first stressed the fundamental role of dissipation and fragmentation in gravitational collapse[7]. It is convenient to discuss separately the problems of star and galaxy formation.

Consider a collapsing primordial gas cloud, which can initially be neutral and consist of hydrogen and helium. The cloud will heat up until at $\sim10^4$ K it can cool by excitation of Lyman α-photons, and will continue to collapse approximately isothermally. While the collapsing cloud can radiate freely, it will be gravitationally unstable to fragmentation on smaller and smaller scales, until eventually the individual fragments become opaque either to electron scattering or to H^- opacity. The subsequent, quasi-adiabatic collapse of what one may now identify as protostellar fragments, will be stable against continued fragmentation, provided that the collapsing fragments individually undergo a luminosity-controlled Kelvin–Helmholtz contraction.

To maintain stability, a fragment must be unable to radiate away freely the compressional energy acquired by collapse. The collapse will proceed with $\gamma \approx 1$, and will be unstable to small perturbations: this should manifest itself, however, as convection or turbulence because the ensuing pressure gradients will have a strong stabilising influence on any tendency to further subfragmentation. Sufficiently massive fragments will, however, undergo free-fall, and in this case fragmentation can continue until smaller fragments form that are themselves pressure-supported. This condition can be expressed quantitatively by requiring that the Kelvin–Helmholtz contraction time of a fragment exceed its free-fall time, when the gas temperature is ~0.1 Rydberg (characteristic of hydrogen atom excitations by electron and atom collisions). The Kelvin–Helmholtz contraction time for a protostar of primordial composition, mass M, and luminosity L is $t_{KH} \sim M(kT/\mu)L^{-1} \sim (k/\mu)^5 T\sigma_T G^{-4}(m_p\sigma)^{-1}M^{-2}$, where μ is the mean molecular weight g^{-1}, σ is the Stefan–Boltzmann constant, σ_T is the Thompson cross-section, and T is the central temperature. Since the free-fall time $t_{ff} \sim (G\rho)^{-1/2} \sim GM(kT/\mu)^{-3/2}$, the condition $t_{KH} > t_{ff}$ is equivalent to $M < (k/\mu)^{13/6}T^{5/6}G^{-5/3}(\sigma_T/m_p\sigma)^{1/3}$. One consequently infers that this is equivalent to imposing an upper limit on the mass of a protostar of $N_p \sim \alpha^{7/3}\alpha_g^{-5/3}$ nucleons or about $\sim50\ M_\odot$. For conventional interstellar matter, where the relevant temperature ($\sim10^{-3}$ Rydberg) is determined by the melting of refractory grains, this limiting mass is approximately $0.2\ M_\odot$[8]. Thus typical stars forming now should have relatively low mass (although other effects, notably due to magnetic fields, may enable more massive stars to form), whereas primordial stars are likely to be massive stars.

The mass of a stable nuclear-burning star is known[1-4] to be of order $N_* \sim \alpha_g^{-3/2}$. Apart from numerical factors, less massive stars have degenerate cores, and more massive stars are dynamically unstable. Thus if gravitational fragmentation is to lead to star formation, one evidently requires $N_* \ll N_p$, or $\alpha_g \ll \alpha^{14}$. This upper bound on $\alpha_g (\sim 10^{-30})$ demonstrates the weakness of the gravitational interaction, as determined solely by the requirement that protostellar collapse leads to the formation of stable nuclear-burning stars. If this inequality were violated, stars may still form (compare ref. 9) but would not have undergone a pressure-supported core accretion phase. Such stars may differ drastically from conventional stars; for example, condensation of planets may be inhibited (compare also ref. 2).

Consider next the collapse of a galaxy from an ionised protogalactic gas cloud of arbitrary size but of some specified mass. Such a cloud must initially collapse in adiabatic free-fall, its cooling time ($\propto \rho^{-1}$) being long compared with its free-fall time ($\propto \rho^{-1/2}$). At the instant when the local cooling rate overtakes the collapse rate, if the cloud is moderately inhomogeneous, it will become unstable to fragmentation, and erupt in star formation. The hydrogen cooling rate, due mostly to free–bound transitions at $kT \lesssim 1$ Rydberg, can be expressed as $t_{\mathrm{cool}}{}^{-1} \propto \rho T^{-3/2}$; hence the mass of a gas cloud in gravitational equilibrium satisfies $M \propto \rho^{-1/2} T^{3/2} (t_{\mathrm{cool}}/t_{\mathrm{ff}})$. Since fragmentation into stars proceeds at $T \sim 0.1$ Rydberg, clouds more massive than N_{gal} (defined by $t_{\mathrm{cool}} = t_{\mathrm{ff}}$) must form stars at a much slower rate (by eventually cooling to ~ 0.1 Rydberg). This necessarily qualitative argument suggests that there is a critical mass $N_{\mathrm{gal}} \sim \alpha^5 \alpha_g{}^{-2} (m_p/m_e)^{1/2}$ ($\sim 10^{12}\, M_\odot$) below which clouds can cool within a free-fall time: larger, more slowly evolving, clouds cool relatively slowly and are liable to subfragmentation or disruption by collisions with neighbouring clouds. One infers that N_{gal} is a characteristic mass for galaxies. (One can also show that associated with this mass-scale is a characteristic dimension of 50 kpc.) Since the number of baryons in the observable Universe is of the order $N_u \sim \alpha_g{}^{-2}$ (ref. 3), one infers that the number of observable galaxies should be of the order $N_u/N_{\mathrm{gal}} \sim \alpha^{-5}$, whereas the number of stars in a galaxy is $N_{\mathrm{gal}}/N_* \sim \alpha^5 \alpha_g{}^{-1/2}$. It is amusing that these numbers only coincide if $\alpha_g \sim \alpha^{-20}$.

Introduction of certain cosmological considerations can further our speculations. At present, the Universe is well described by the Friedmann–Lemaitre cosmology, and observations of the microwave background radiation reveal the homogeneity and isotropy of the Universe at early epochs. In such a cosmological framework, primordial curvature fluctuations play an essential role in accounting for large-scale deviations from homogeneity. The scale of initial amplitudes of these fluctuations is not relevant; it suffices to say that the Universe is observed to be lumpy on the scales of galaxies, galaxy clusters and superclusters, and reasonably smooth on much larger scales.

A natural length scale for large-scale structure arising out of the early Universe is the maximum Jeans mass (N_J) which is achieved at the epoch when matter and radiation densities are equal. Shortly thereafter, matter and radiation decouple, and the Jeans mass is drastically decreased. Any disturbances in the radiation era (due to inhomogeneities of finite spatial extent) on scales smaller than N_J will act as a diverging source of waves that propagate out to N_J (in much the same way as water waves are produced by dropping stones into a pond). Neighbouring regions of the Universe similarly develop structure on scale N_J, with the result that structure inevitably develops over the maximum Jeans length in the early Universe (provided one has a sufficiently general form of primaeval inhomogeneity). Fragmentation into galaxies can only occur after matter (density ρ_m) and radiation (ρ_r) have decoupled (at $T \sim 0.02$ Rydberg). One has $N_J \sim c^3 t (G m_p)^{-1}$ when the Universe is still radiation-dominated, with t determined by $\rho_m + \rho_r \sim G^{-1} t^{-2}$, and $\rho_r \sim (kT/hc)^3 (kT/c^2)$.

Consequently, N_J can be expressed in terms of fundamental constants by adopting a temperature equal in order of magnitude to a Rydberg, yielding $N_J \sim S^{1/2} \alpha_g{}^{-3/2} \alpha^{-3} (m_p/m_e)^{3/2}$, or $\sim 10^{16} (S/10^8) M_\odot$. Here the mean entropy per baryon $S \sim 10^8$ is equal to the number of cosmic blackbody radiation photons per hydrogen nucleus and is approximately constant during the course of the nearly isentropic cosmological expansion. We thus account for the order of magnitude of S by noting that N_J must at least be comparable in mass to a supercluster of galaxies. This suggests that the entropy of the Universe is causally related to its large-scale structure.

Strictly speaking, this argument provides only a lower limit on S for an assumed value of α_g; inhomogeneities on scales larger than superclusters could also exist. An upper limit on S is found by requiring that the dynamics of the Universe be matter-dominated (thereby enabling gravitational instability to proceed, to allow formation of galaxies and stars) when the age of the Universe is first comparable with the lifetime of a star. To evaluate this condition, one can set $t = \alpha_g{}^{-1}(h/m_p c^2)$ in the above expression for ρ_r, yielding $kT/m_p c^2 \sim \alpha_g{}^{1/4}(1+\rho_m/\rho_r)^{-1/4}$. Since $S = (\rho_r/\rho_m)(m_p c^2/kT)$, one concludes that, if $\rho_r < \rho_m$, one must S have $\lesssim \alpha_g{}^{-1/4}$. Thus, knowledge of α_g is essential for determining S.

A lower bound on α_g can be obtained independently of S by noting that for galaxies to exist, larger primordial inhomogenities must be present, whence $N_J \gg N_{\mathrm{gal}}$. This condition yields $\alpha_g \gg \alpha^{16} (m_e/m_p)^2 S^{-1}$, and using the upper bound on S just given, one concludes that $S^{-4} \gtrsim \alpha_g \gg \alpha^{64/3} (m_e/m_p)^{8/3} \approx 10^{-44}$. Therefore the finite entropy of the Universe strongly constrains α_g, and is intimately related to the weakness of the gravitational interaction.

The formation of galaxies and stars provides an insight into the magnitude of α_g. Moreover, it seems plausible that the existence of large-scale structure can only occur in a universe with sufficiently great entropy per baryon. Thus one returns (perhaps inevitably) to a modified form of the anthropic hypothesis: gravitational instability and fragmentation must lead from giant clusters to galaxies to stars, and ultimately to planets and an environment suitable for the development of life. This unbroken chain is essential in any cognisable universe, and may therefore provide the key to understanding the significance of the fundamental dimensionless numbers of astrophysics and cosmology.

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A method for determining the cosmological deceleration parameter q_0

THE availability of deep plates from the UK 48-inch Schmidt combined with the ability to measure the apparent magnitude of large numbers of images using the Royal Observatory Edinburgh COSMOS machine has made possible a new experiment to determine the cosmological deceleration constant q_0. We have constructed a number–magnitude plot for a large sample of images and compared this with a number of simulated curves representing different values of q_0

The coordinates of the plate centre were: right ascension 2 h 41 min 00 s, declination −30°00′ which is a field near the South Galactic Pole. The plate was exposed for 60 min on December 8, 1974, using a combination of unsensitised Eastman Kodak 098 emulsion and a Schott RG630 filter and was measured on the Royal Observatory Edinburgh COSMOS machine using the coarse measurement mode (CM) (ref 1). The total area of the plate measured was 12.5 × 13.5 cm and the plate scale was 67 arc s min^{-1}.

The CM mode measures the extents, centroids, blackness and area of the images above the plate background. COSMOS currently scans in units of 8 μm using a 25-μm spot full width half height. The image area, A, of principal interest in this experiment is digitised in increments of 8 μm^2.

A is a measure of the area of an image above the computed sky background. The relationship between log A and apparent magnitude m has been investigated[2]. These results, from a number of Schmidt plates, show the relationship to be linear, of the form

$$m = b \log A + \text{const} \qquad (1)$$

throughout the range $1.0 \leqslant \log A < 2.2$. The parameter b varies slightly from plate to plate, as may be expected, and in the present case, $b \approx 3.5$.

A total of 41,225 images was detected above the plate threshold but only images of 10 increments or greater were included in the analysis. This corresponds to a minimum image size of 1.77 arc s while the plate was taken in seeing of ~ 1 arc s. The number of images N, in each digitised area bin A was plotted logarithmically in Fig. 1.

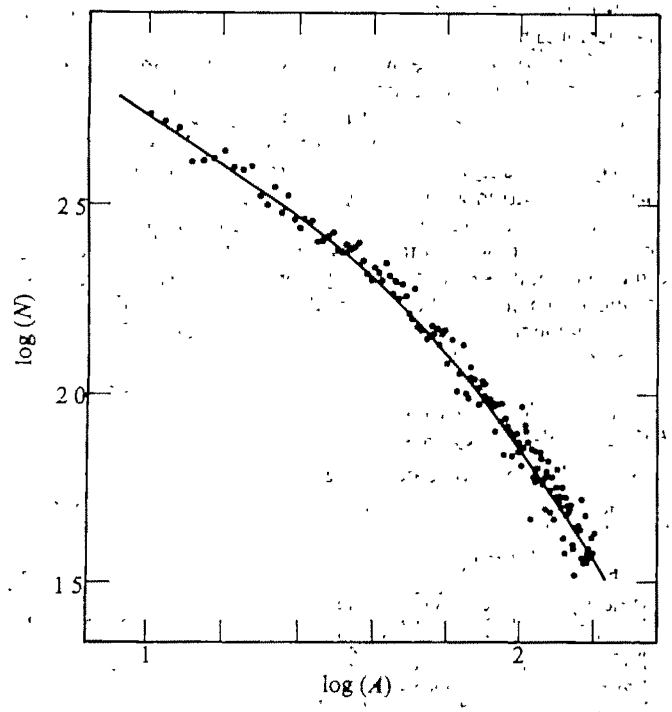

Fig. 1 Log (N)–log A relationship showing best fit curve to observed data. Dots represent number of images in each area bin.

A preliminary examination of the log N–log A plot in Fig. 1 shows a noticeable change in slope in the region of log A = 1.5. To determine the cause of this a simulated number–magnitude plot was constructed on the basis of the absolute fluxes for galaxies given in ref. 3. The procedure adopted was as follows. k-corrections were evaluated by computer for each galaxy type with the effective wavelength bandpass given by the filter–photographic plate combination described above. The k-correction is applied to the apparent magnitude to compensate for the Doppler shift of the spectrum across the filter combination and is a function of the galaxy type. It is defined by

$$k(z) = 2.5 \log(1+z) + 2.5 \log \left(\frac{\int_0^\infty F(\lambda) S(\lambda)\, d\lambda}{\int_0^\infty F\left(\frac{\lambda}{1+z}\right) S(\lambda)\, d\lambda} \right) \qquad (2)$$

where F is the intrinsic flux distribution of the galaxy as a function of wavelength and S is the effective detector response.

Mattig[4] has shown that a galaxy of absolute magnitude M at redshift z will have an apparent magnitude m, which, with the addition of the k-correction, is given by

$$m = M + 5 \log D(z) + k(z) + \text{const} \qquad (3)$$

where $$D(z) = \frac{1}{q_0^2} \left\{ q_0 z + (q_0 - 1)(\sqrt{(1+2q_0 z)} - 1) \right\} \qquad (4)$$

and q_0 is the deceleration parameter.

The predicted number of galaxies of magnitude m is calculated by computing the volume of space at that redshift. This is given by Mattig as

$$N = \begin{cases} (1-2q_0)^{-3/2}\,(\varphi\sqrt{(1+\varphi^2)} - \text{arc sinh}\,\varphi) & q_0 < \frac{1}{2} \\ 16/3\ D^3(1+D+\sqrt{(1+2D)})^{-3} & q_0 = \frac{1}{2} \\ (2q_0-1)^{-3/2}\,(\text{arc sin}\,\varphi - \varphi\sqrt{(1-\varphi^2)}) & q_0 > \frac{1}{2} \end{cases} \qquad (5)$$

where $$\varphi = \frac{D\sqrt{|2q_0 - 1|}}{q_0(1+D) - (q_0-1)\sqrt{(1+2D)}}$$

(D defined as in equation (4)).

This calculation is repeated for a range of values of z. The frequency distribution of the absolute magnitudes of galaxies of a particular type is believed to be Gaussian. This luminosity function is given by Pence[3] for various galaxy types.

The calculation of the apparent magnitudes was repeated for each galaxy type throughout the range of absolute magnitude, and the total number of each apparent magnitude was then weighted according to the luminosity function. The entire process was repeated for each galaxy type to give the overall distribution of apparent magnitudes.

Thus the predicted relationship between log N and m was obtained for a given q_0. The simulated curves showed a change in slope over a range of values of q_0. This is attributable to a marked change in the value of the k-correction due to a broad absorption feature being red-shifted into the filter combination. This suggested the possibility of fitting the simulated data to the observed by varying the value of q_0 in the simulation. To achieve this, the simulated log N–m relationship was evaluated for values of q_0 in the range $0 \leqslant q_0 \leqslant 2$ in intervals of 0.1. To determine the minimum r.m.s. difference between the observed and the simulated curves it was necessary to fit for zero points of the simulated log N and m axes since these were not included in the simulation. This process provided a best fit value for q_0. This value, however, was somewhat dependent on the scaling factor of the magnitude axis which had been included in the simulation (variable b to equation (1)). Therefore, due to the uncertainty in b it was decided to fit this parameter as well. As a crosscheck, we tested the consistency of the method by fitting for a scaling factor in the log N axis.

The fitting was done by a combination of analytic and iterative computer processes for each value of q_0; this is equivalent to laying the simulated data on the observed, and aligning and stretching the axes in order to give the minimum r.m.s. deviation between the two curves.

The minimum r.m.s. for each value of q_0 is plotted in Fig. 2. There is a minimum in the region of $q_0 \simeq 0.8$. The solid line represents the best fit cubic polynomial to these points, which has its minimum value at $q_0 = 0.81$. The formal s.d. in the fit is $q_0 = \pm 0.04$. A more realistic error is obtained, however, by taking the limits defined by applying the r.m.s scatter about the best fit to the minimum point which gives $q_0 = 0.8 \pm 0.3$. The simulation corresponding to $q_0 = 0.8$ is plotted as a solid line in Fig. 1.

The magnitude scaling factor obtained from the best fit was $b = 3.45$, which compared favourably with the predicted value 3.5. The log N scaling factor was expected to be 1.0. Evolutionary effects would tend to increase this value, however, implying that objects were either more numerous or brighter in the past. The value obtained was 1.05, which does not differ far enough from unity to indicate a significant evolutionary effect. The smallness of the deviation from unity indicates, however, that the simulation was an adequate model for the observed distribution.

The possibility of effects other than the *k*-correction (for example, some data acquisition error) were examined to see if they were capable of causing the shape of the observed distribution.

Extensive tests carried out with COSMOS recently indicate that for image areas greater than 10 increments the systematic errors in the measured areas are small. The smallest images used in the analysis are at least one magnitude above the plate limit and the critical change in slope is approximately 2½ magnitudes above the plate limit. Photographic effects such as reciprocity failure have negligible effect on the image size at this level. A possible source of error is the construction of the model used in the simulation. It is now generally accepted that galaxies were intrinsically brighter in the past[5]. This means that any method of evaluating q_0 which relies on measuring apparent magnitudes should make allowances for evolutionary effects, which tend to increase the measured value of q_0. This has been discussed in recent papers in which the magnitude–redshift relationship is used to evaluate q_0[6], but as the evolutionary processes involved remain uncertain, it was decided to exclude them from this model. Similarly, effects due to intergalactic absorption, which would tend to decrease the measured value of q_0, but which also are uncertain, have also been ignored. However, the proximity of the log *N* scaling factor to unity gives weight to the argument that the sum of these effects is small.

One possible source of error in the model was the absence of stars in the simulation although some stars will appear in the observed data. It may be deduced from recent observations of the stellar luminosity function near the South Galactic Pole that the proportion of stars to galaxies in the observed data is probably small[7,8]. Nevertheless, an attempt was made to incorporate stars into the simulated model, but the effects were small. The value of q_0 could be reduced by up to ~0.4, but at present there is a large uncertainty in the stellar luminosity function at these magnitudes. Therefore for the final result the stars were excluded. It should be pointed out, however, that a proportion of the images in the observed data were of 'stellar' appearance. It is probable that these objects are predominantly distant galaxies where only the compact central region is observed, as would be expected from known galaxy profiles.

Again, the derived scaling factor of the log *N* axis indicates that there was no appreciable contamination of the data by stars.

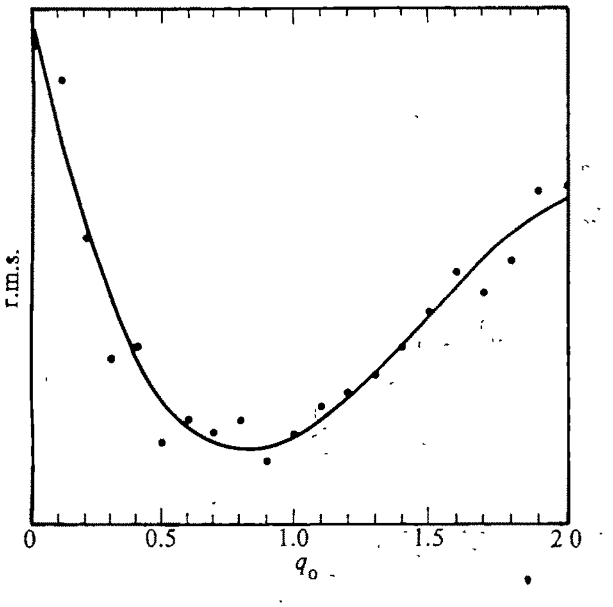

Fig. 2 r.m.s. deviation of best fit curve for each value of q_0 from the observed data in Fig. 1.

The result of this experiment gives a formal value

$$q_0 = 0.8 \pm 0.3$$

It should be possible to estimate the size of some of the possible systematic errors by further analysis of independent samples. We intend to repeat the experiment with deeper plates and a calibration system that is currently under development.

This experiment has the advantage that the observed data may be assumed to contain a complete sample of intrinsic galaxy types throughout the redshift range considered. This means the estimated value of q_0 is independent of the Hubble constant and the absolute magnitude of the galaxies.

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Sunspot cycles and solar activity forecasting

I REPORT here that there is a linear relation between the relative intensity of solar cycle activity, as measured by the ratio (μ) of maximum annual mean sunspot number of two consecutive cycles, and the relative skewness of the first cycle. This relationship facilitates the forecasting of the intensity of the next cycle towards the end of a current cycle. The solar cycle just starting is likely to be more active, with an expected maximum annual average sunspot number of 195, compared with 106 for the cycle just ended.

The solar cycles as represented by the mean annual sunspot number for the period of observations beginning in the year 1756 reveal no obvious pattern in the variations of solar cycle activity as measured by the maximum annual sunspot number. The cycles are all asymmetrical. The degree of asymmetry of the cycles may be represented by the statistical parameter of relative skewness (β), where

$$\beta = \frac{m_3}{\sqrt{(m_2^3)}} \qquad (1)$$

and m_3 is the third moment about mean, or $(\Sigma f_i x_i^3)/\Sigma f_i$, with f_i the frequency and x the deviation about the mean; $m_2 = S^2$ is the second moment about mean the S and the standard deviation.

In general, the greater the sunspot activity, the more positive the cycle skewness; this is illustrated in Fig. 1. Cycles with R_{max} (maximum annual mean sunspot number) less than 100 have $\beta < 0.35$ (with one exception), and cycles with higher R_{max} have $\beta > 0,35$ (with two exceptions). In Fig. 2 the β values of the cycles are plotted against the ratio μ of the R_{max} of the next

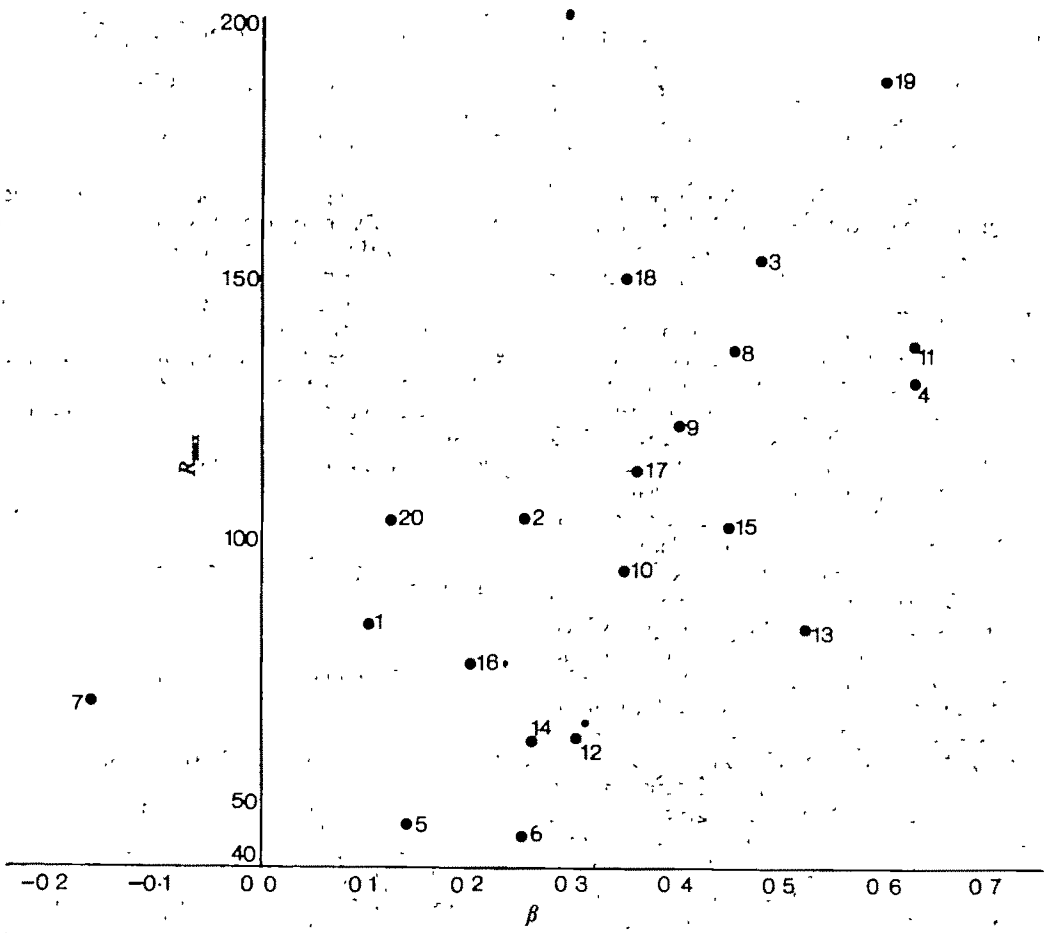

Fig. 1 R_{max} of cycle (mean annual sunspot no.) at various values of cycle skewness (β). Numbers against points are cycle numbers from cycle 1756–1766.

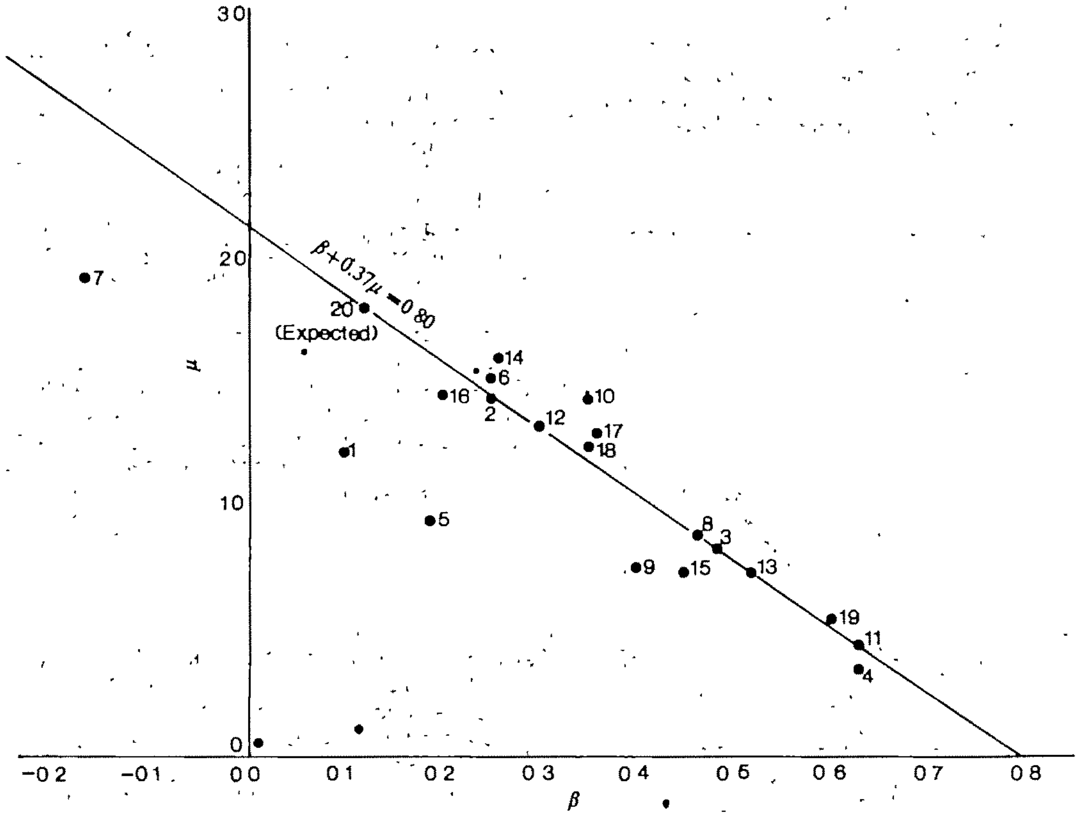

Fig. 2 Ratio of R_{max} of next cycle to R_{max} of current cycle (μ) for various values of β Numbers against points are cycle numbers from cycle 1756–1766.

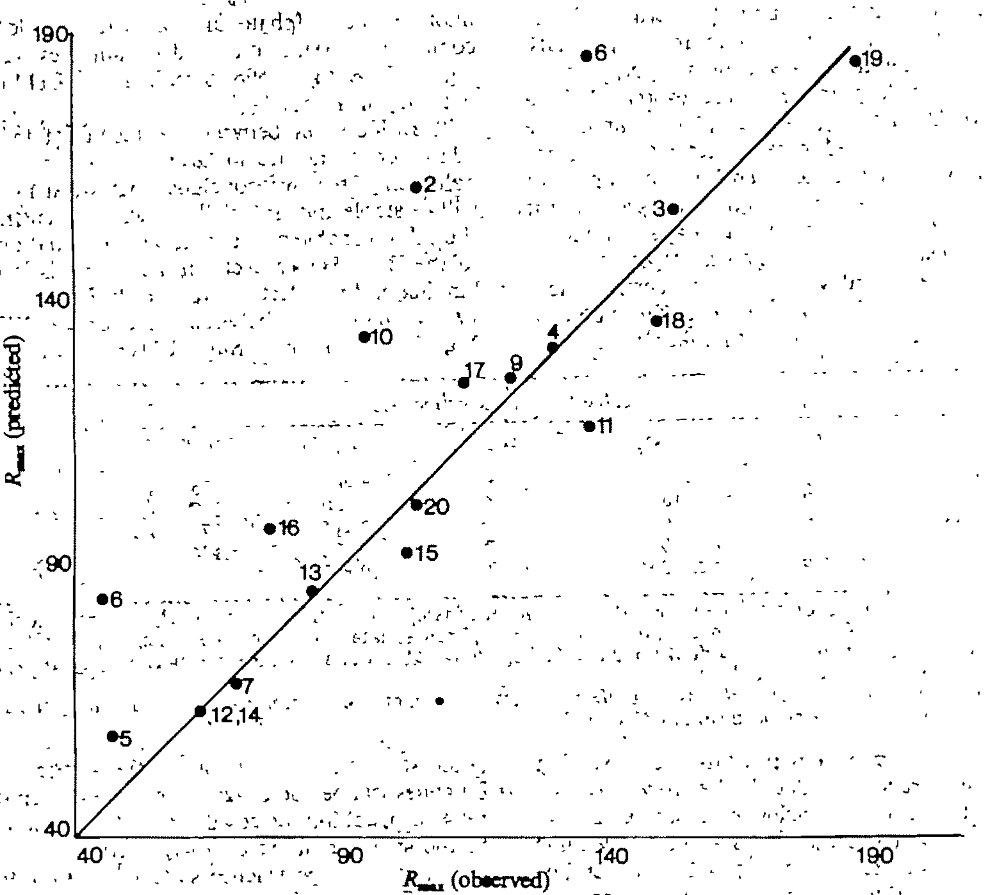

Fig. 3 Predicted and observed values for $R_{\max}$. Numbers against points are cycle numbers from cycle 1756–1766.

cycle and $R_{\max}$ of a current cycle. This plot exhibits linearity and may be represented as

$$\beta + 0.37\,\mu = 0.8$$

Figure 3 is a scatter plot of the observed $R_{\max}$ values of the cycles against the $R_{\max}$ values derived from the above linear relation. The scatter about the equidistant line is less than 15% for all but five of the 19 cycles plotted and 50% of the cycles show less than 5% error.

The above empirical relation was established in 1965 (G.R., unpublished thesis), and verified for cycle 20 just ending. Extending it to the coming 21st cycle, it seems that this cycle should be very active. The formula predicts that activity will increase by 1.84 times the activity of the previous (20th) cycle. The expected intensity may thus be close to that of the 19th cycle which was the most active in 200 yr. In 1978, or within 3 yr of the onset of the new cycle, this sunspot high may be observed.

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Theoretical chemistry of superheavy elements E116 and E114

RECENT evidence[1] for the presence of primordial superheavy elements 126, 124, 116 and possibly 114 in microscopic crystalline monazite inclusions in biotite micas raises several interesting questions. Earlier work[2,3] suggested reasons why E124 and E126, the homologues of U and Pu, respectively, might occur in such an environment, but gives little information about E116. Better information on both E114 (eka–Pb) and E116 (eka–Po) could enable us to see why they might occur in these minerals and help suggest methods of concentration. At present, *ab initio* relativistic structure calculations for molecules containing superheavy elements are not feasible. We have developed techniques, however, for studying the structure of heavy atoms and ions using the multi-configuration Dirac–Fock (MCDF) relativistic self-consistent field method[4]; this is a considerable advance on the single configuration Dirac–Fock or Dirac–Slater techniques previously available[2,3]. Applications of the MCDF method to atoms and ions of Ba, Hf (Ref. 4), Bi and U, for example, have yielded good ionisation potentials and energy spectra, mainly due to the way in which relativistic effects, which increase dramatically in size with atomic number[2–4], are handled. These effects make it difficult to predict some chemical properties of superheavy elements by extrapolation from the Periodic Table. We use here, therefore, the results of MCDF calculations of some properties to estimate the heat of formation of simple compounds of E114 and E116 with the simple ionic model[5] and with Pauling's semi-empirical valence-bond resonance model[6], to acquire insight into the chemistry of these elements.

The atomic data required are listed in Table 1. Ionisation potentials (IP) were obtained from energy differences of MCDF ground states of the relevant ions, whenever no reliable experimental value was available, adding an energy of 77 kJ mol^{-1} to correct for correlation effects not allowed for in the calculation[2,3]. Ionic radii were estimated from the mean radius of the outermost Dirac–Fock orbital using a method to be reported elsewhere; for E114^{+2}, this estimate differs by 0.01 Å from the value linearly extrapolated from Sn^{2+} and Pb^{2+}. Lattice energies were estimated from the Kapustinskii equation (ref. 5), taking $\rho = 0.345$ in the Born–Mayer repulsion formula. The overall uncertainty in the lattice energy is too small to affect our conclusions; we shall report the details of our investigations elsewhere. Heats of sublimation, if not available, were estimated by extrapolating down the relevant group of the Periodic Table; their contribution is dominated by the ionisation potentials, and extrapolation errors are unimportant for our purposes.

The data for the covalent model[6] were estimated in a similar way. The MCDF program was used to compute the energy, E_v, needed to excite a superheavy atom to a valence state which can form strong bonds. This is an important contribution for E116 and E114, whose ground states are almost pure jj coupled; such states contain a substantial anti-bonding component[6]. Homonuclear bond strengths, *D*(X–X), were estimated following Phillips and Williams[7], and heteronuclear bond strengths, *D*(X–Y), from Pauling's formula[8]. The unknown electronegativities, χ_v, were found from Mulliken's formula[8], using MCDF estimates of ionisation potentials and electron affinities. Again, we shall report the details elsewhere.

Table 1 Atomic properties

Atom	r_{+2} (Å)	r_{+4} (Å)	ΔH_s	IP_2	IP_4	E_v*	χ_v	*D*(X–X)
Te	1.28	0.94	197	2,665	8,966	75	2.1	138
Po	1.32	0.97	142	2,364	8,640	197	1.8	105
E116	1.35	1.01	109	1,937	8,711	577	2.0	79
Pb	1.21	0.84	197	2,167	9,330	134	1.8	109
E114	1.29	0.91	126†	2,531	10,247	427	1.6	96

Heats of sublimation (ΔH_s), ionisation potentials (IP_2/IP_4 for removal of two/four electrons, respectively), valence excitation energies (E_v) and homonuclear bond strengths (*D*(X–X)) are in units of kJ mol^{-1}. Experimental data have been used, when available, for Te, Po and Pb.

* E_v is the promotion energy for divalent p-orbital bonding. The sp^3 tetrahedral quadrivalent state of E114 requires a promotion energy of 1,665 kJ mol^{-1}, making such compounds unlikely.

†This is probably an upper bound. Keller *et al.*[2], using a different extrapolation, give 42 kJ mol^{-1}, which would make the predictions of ΔH_f for E114 compounds in Tables 2–4 more stable by about 84 kJ mol^{-1}.

Some care is needed in drawing conclusions from Tables 2–4. We use enthalpy instead of free energy to assess thermodynamic stability; however, the difference is too small to affect our conclusions at room temperatures[11]. An endothermic compound ($\Delta H_f > 0$) is not necessarily unstable (e.g. H_2Te). An exothermic compound ($\Delta H_f < 0$) is stable against decomposition to the free elements, but can still be unstable with respect to other decomposition or disproportionation reactions. It seems reasonable to predict that a compound is definitely covalent or ionic if the difference in the respective values of ΔH_f is sufficiently large, say 200 kJ mol^{-1}.

Table 2 Predicted heats of formation of dihalides (kJ mol^{-1})

	Ionic model				Covalent model (gas phase)				
Atom	F^-	Cl^-	Br^-	I^-	F	Cl	Br	I	H
Te	−79	297	410	598	−561*	−272	67	163	134
Po	−397	−29	79	264	−699	−46	67	201	213
E116	−837	−473	−335	−155	−163	418	514	623	611
Pb	−628	−238	−121	71	−707	−54	59	192	205
E114	−272	100	218	402	−644	84	209	360	406

*Covalent TeF_2 is unknown, presumably because it is unstable with respect to disproportionation to covalent TeF_4+Te.

Thus, we predict that E114 should be less reactive than Pb, mainly because IP_2 is 313 kJ mol^{-1} larger than one would expect by extrapolating from Sn and Pb. Other contributions to ΔH_f vary smoothly down the group, so that this relativistic atomic effect dominates. The ionic–covalent resonance term, which may here be slightly overestimated, is responsible for the relatively large covalent energy of $E114.F_2$. Although the gaseous halides, other than the fluoride and, possibly the chloride (Table 2 footnote) seem to be endothermic, the ionic fluoride and sulphate are predicted stable, contradicting a conjecture[6], based on cruder estimates (E_v(Pb) = 192 kJ mol^{-1}, E_v(E114) = 598 kJ mol^{-1}), that E114 might behave as a relatively inert gas.

Table 3 Predicted heats of formation of divalent inorganic salts using the ionic model (kJ mol^{-1})

Atom	SO_4^{2-}	CO_3^{2-}	NO_3^-	PO_4^{3-}
Te	−226	−21	180	−347
Po	−552	−343	−151	−1,318
E116	−971	−782	−569	−2,641
Pb	−761	−569	−356	−1,983
E114	−418	−213	−17	−929

The differences between the results of the two models for E116 compounds are so large, and so different from those for polonium, that we conclude that divalent halides of E116 will be stable and essentially ionic, and that the salts of Table 3 should be capable of preparation. Examination of contributions to the Born–Haber cycle shows that this is due to the weak binding of $7p_{3/2}$ electrons (ε = 632 kJ mol^{-1}); the value of IP_2, 1937 kJ mol^{-1}, lies between those for Ca (1736 kJ mol^{-1}) and Mg (2188 kJ mol^{-1}). Since $E116^{2+}$ has a larger ionic radius (1.35 Å) than Mg^{2+} (0.68Å), the lattice energies of E116 dihalides will be about 420 kJ mol^{-1} less than those of Mg. The relative instability of covalent E116 compounds is mainly due to the strong binding of $7p_{1/2}$ electrons (ε = 1414 kJ mol^{-1}) leading to a large valence state promotion energy of 577 kJ mol^{-1}. The E_v and χ_v are larger than expected from extrapolation down Group VIB.

The reliability of our models for E116 can be assessed from the corresponding results for Te and Po. We conclude that tellurium dihalides are all covalent, in agreement with known chemical properties, whilst $TeCO_3$ and $Te(NO_3)_2$ are almost certainly unstable with respect to the oxide, and complex tellurium sulphates are known[10]. Similarly, the emergence of cationic properties for polonium is correctly predicted.

The ionic radius of $E116^{4+}$ (1.01Å) and the energy required to ionise the two $7p_{1/2}$ electrons (6774 kJ mol^{-1}) are larger than those of Po^{4+} (0.97 Å and 6276 kJ mol^{-1}), making its quadrivalent ionic compounds less stable than those of polonium. Whilst polonium forms volatile $PoCl_4$, $PoBr_4$ and PoI_4, which are presumably covalent in the gas phase, the E116 analogues seem unlikely to be stable. The covalent dihalides seem to be strongly endothermic, and the energy released by forming two more bonds would not be sufficient for stability when the promotion energy of a p^3d valence state is taken into account. Hexavalent E116 compounds seem unlikely to be stable because the $7s$–$7p_{3/2}$ energy gap (2063 kJ mol^{-1}) is much larger than the corresponding $6s$–$6p_{3/2}$ gap (1330 kJ mol^{-1}) in polonium.

We conclude that the chemistry of E116 should be mainly cationic; although it is in Group VIB, we expect it to form divalent ionic compounds with an ease approaching that of beryllium or magnesium, and quadrivalent compounds with the most electronegative atoms. The element might reside in monazite as

Table 4 Predicted heats of formation of quadrivalent compounds using the ionic model (kJ mol^{-1})

Atom	F$^-$	Cl$^-$	Br$^-$	I$^-$	SO$_4^{2-}$	CO$_3^{2-}$	NO$_3^-$	PO$_4^{3-}$†
Te	−900	552	966	1,619	−92	−193	397	233
Po*	−1,180	326	653	1,243	−418	−485	88	−682
E116	−908	377	774	1,351	−297	−339	213	−285
Pb	−866	678	1,109	1,732	377	−113	531	594
E114	218	1,695	2,113	2,715	1,042	937	1,540	3,653

*The sulphate, carbonate and phosphate of polonium are all known experimentally.

†Neglects the unknown heat of formation of the gaseous phosphate ion. Attempts to estimate this quantity from known phosphates gave numbers ranging from +8 to −364 kJ mol^{-1}.

a divalent phosphate or as a quadrivalent phosphate or mixed oxide, the sizes of E116^{4+} and Ce^{4+} being very similar. The presence of small quantities of Ca^{2+} and Mg^{2+} ions in the mineral has been noted[11].

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Vacuum ultraviolet radiation scales

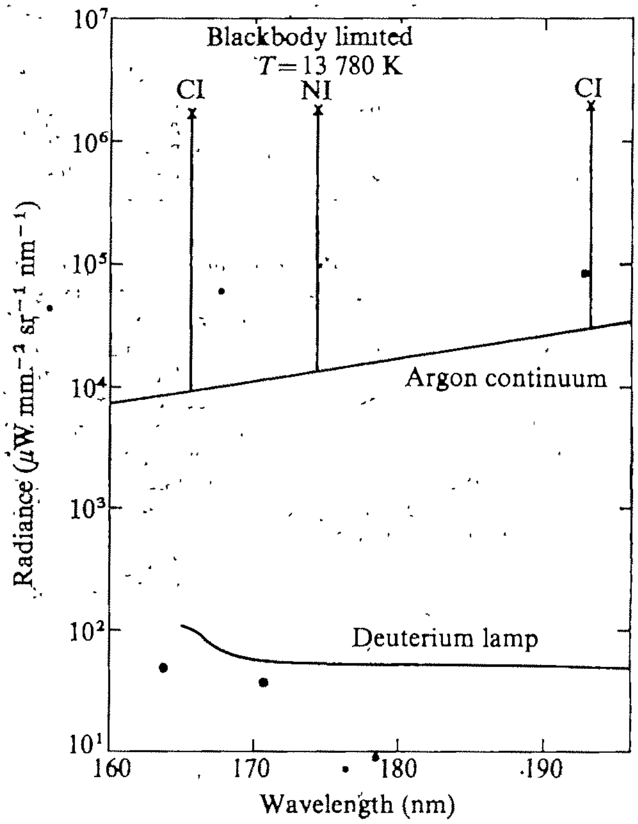
WE report here a comparison of two independent techniques (high-temperature argon arc and synchrotron radiation) for the determination of vacuum ultraviolet spectral radiances. Basic agreement between the methods has previously been demonstrated at 165.7 nm by Stuck and Wende[1], but we have refined the techniques to produce significant improvements in accuracy and have extended the comparison to other wavelengths.

The addition of trace gases to a high-temperature wall stabilised argon arc results in the production of optically thick resonance lines (for example from C, Kr, N, O) at wavelengths predominantly in the vacuum ultraviolet[2]. The determination of the blackbody limited spectral radiances at the centres of these lines requires only a measurement of plasma temperature. Recent research at NPL (R.C.P., in preparation) has produced increased accuracy in such temperature determinations (±55 K at 13,800 K) as a result of the application of various plasma diagnostic techniques. There have also been refinements in techniques for producing saturation of the resonance line radiation with minimal perturbation to the plasma, and in correcting for the effects of the residual perturbation. As a result, resonance line radiances can be established with a marked improvement in accuracy, for example ±3.2% at 165.7 nm and ±2.6% at 193.1 nm.

The method based on the predictable spectral emission properties of synchrotron radiation[3,4] is well established. In the past few years we have used several different experimental techniques, with both the Glasgow University and the SRC Daresbury synchrotrons, to set up an accurate ultraviolet spectral emission scale (P.J.K., in preparation), initially down to 200 nm. The synchrotron radiation has been used only as a standard of relative spectral distribution, since the circulating electron current cannot be measured with sufficient accuracy to establish absolute levels. These are therefore set by comparison at about 350 nm with existing visible/near-ultraviolet standards based on conventional blackbody radiators. Work at Daresbury has extended this scale into the vacuum ultraviolet, currently to a limit set at about 165 nm by the availability of suitable transfer standard sources. These are normally deuterium discharge lamps, producing continuum radiation from 165 to 370 nm, and are specially modified and selected versions of commercially available lamps, with ageing rates of less than 0.05% per h at all wavelengths. The accuracy now attainable in the calibration of these sources is estimated as ±2.7% at 200 nm and ±3.6% at 165 nm.

To effect the intercomparison, four deuterium lamps were calibrated using synchrotron radiation and their spectral radiances then determined by the argon arc method at the three resonance lines, 165.7 nm (CI), 174.3 nm (NI) and 193.1 nm (CI). The measurements were made with a very high resolution concave grating monochromator to investigate adequately the saturation of the peak of the blackbody resonance lines. The aperture ratio of the optical system (f/260) was determined by the aper-

Fig. 1 Radiances of resonance lines, argon continuum and a typical deuterium lamp.



tures in the differential pumping system and by the need to restrict the field of view to regions of adequate temperature uniformity. The arc was 55 mm long and run at 60 A in argon at a pressure of 1.75×10^{5} Pa, producing an axial temperature of 13,780 K. The radiance levels of the line peaks, the argon continuum and a typical deuterium lamp are shown in Fig. 1. Because of the large dynamic range, the intercomparison was made by first measuring the ratio of resonance line peak to argon continuum and then, with an increased exit slit width, the ratio of argon continuum to deuterium lamp radiance.

A major experimental problem arose because the spectral distribution of the deuterium lamp radiation varied significantly from the centre to the edge of the 1-mm diameter emitting area. Synchrotron measurements were based on a 0.9-mm diameter sample, arc measurements on a 0.2×0.03 mm central spot, and detailed investigation revealed correction factors that were large and very wavelength dependent, especially at shorter wavelength (~7% at 165.7 nm). Another difficulty at 165.7 nm was the onset of the complex deuterium molecular line emission spectrum superimposed on the continuum. Different spectral bandwidths in the synchrotron and arc calibration systems required computer-based convolutions of a high resolution deuterium spectrum to reduce radiance measurements to the same bandwidth conditions. Since the calculated correction factors were very sensitive to wavelength, a further measurement of the deuterium lamp emission was made at 167 nm, away from the line emission, by comparison with the argon arc continuum. This continuum level was in turn obtained by comparison with the 165.7-nm line, the change in instrumental response over this wavelength interval being small enough to be measured with adequate accuracy.

The results of measurements on the four lamps are shown in Table 1. The result at 167 nm is based on the 165.7-nm resonance line realisation and so is not totally independent; the difference clearly reflects the problems of the bandwidth and area correction factors. Total intercomparison uncertainties range from 0.9% at 193.1 nm to 2.4% at 165.7 nm and the radiance ratios for all lamps are within the expected limits set by combined intercomparison and basic scale realisation uncertainties. The agreement averaged over the four lamps is extremely good and we conclude that the two methods for establishing vacuum ultraviolet radiances are in agreement to well within their individual scale uncertainties.

Table 1 Ratios of deuterium lamp radiance determined from arc measurements to that from synchrotron measurements

Wave-length (nm)	Lamp no. 786	787	808	810	Mean
165.7	1.009	1.039	1.016	0.993	1.014
167	0.987	1.000	1.004	0.969	0.990
174.3	1.012	0.987	0.998	0.991	0.997
193.1	1.014	0.987	0.995	0.996	0.998

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Volatilisation of methylmercuric chloride by hydrogen sulphide

THE interconversion of mercury compounds in the environment has attracted considerable interest, especially the synthesis and breakdown of the highly toxic methylmercury. The alkylmercurial can be formed and degraded by microorganisms in lakes, rivers, soil and the mammalian intestinal tract[1–6], although the ecological significance of these processes is difficult to assess. We describe here a further reaction which may be important with respect to the fate of mercury in the biosphere—the interaction of methylmercuric chloride (CH_3HgCl) with hydrogen sulphide. Hydrogen sulphide is produced by many bacteria in anaerobic conditions such as are found in the sediments of lakes and rivers, and it has been shown that trimethyllead salts can be converted to the volatile tetramethyllead by reaction with H_2S (ref. 7).

Methylmercuric chloride labelled with ^{203}Hg (Radiochemicals Centre, Amersham), or ^{14}C (New England Nuclear) was purified by extracting it from aqueous solution into benzene, evaporating the organic solvent and redissolving the residue in distilled water.

When solutions of the purified CH_3HgCl were incubated in the presence of H_2S, radioactivity was lost from aqueous solutions within 3–4 d, the rate of loss of radioactivity being similar whether ^{14}C- or ^{203}Hg-labelled CH_3HgCl was used. Analysis of the solutions by thin-layer chromatography revealed that all remaining radioactivity was present as CH_3HgCl. When N_2 or air was used instead of H_2S, or when the bottles were incubated under H_2/CO_2 (97%/3%) in an anaerobic jar (GasPak, BBL Ltd), little or no radioactivity was lost (Fig. 1 and Table 1). Other sulphur compounds, such as cysteine and sodium thioglycollate, did not induce volatilisation of CH_3HgCl and although ammonium sulphide did cause some loss of radioactivity, this may be ascribed to the slight dissociation of the ammonium sulphide with the formation of small

Fig. 1 Volatilisation of CH_3HgCl by H_2S. Solutions of the purified $^{14}CH_3HgCl$ (0.01 μCi μg^{-1}, final concentration 2 μg ml^{-1}) or $CH_3{}^{203}HgCl$ (0.025 μCi μg^{-1}, final concentration 2 μg ml^{-1}) were incubated at 37 °C in screw-cap bottles fitted with rubber septa. H_2S was passed into some of the bottles through hypodermic needles for 2 min before incubation. Samples were taken from the bottles at various times using a syringe and hypodermic needle, so that the atmosphere above the solution was not unduly disturbed. The samples were assayed for residual radioactivity in the solution and for the amount of radioactivity associated with CH_3HgCl by thin-layer chromatography as described previously[4]. ○, $CH_3{}^{203}HgCl$, air; ●, $CH_3{}^{203}HgCl$, H_2S; △, $^{14}CH_3HgCl$, air; ▲, $^{14}CH_3HgCl$, H_2S.

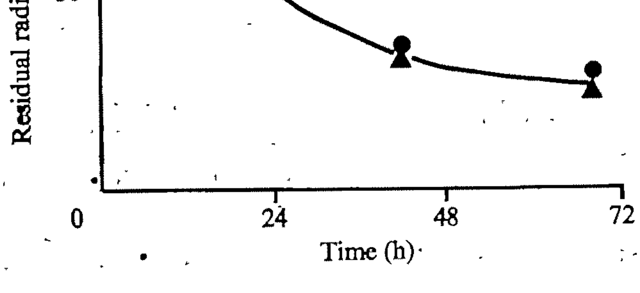

Table 1 Effect of certain gases and sulphur compounds on volatilisation of $CH_3{}^{203}HgCl$ from solution

Compound or gas	Residual radioactivity
Air	98.0±1.2
Nitrogen	97.1±0.9
Carbon dioxide	93.4±1.1
Hydrogen/carbon dioxide (97%/3%)	93.6±2.1
Methane	95.1±1.6
Hydrogen sulphide	30.6±3.0
Sodium sulphide	100.0±0.8
Ammonium sulphide	77.2±1.8
Methionine	97.4±0.5
Cysteine HCl	99.6±1.9
Sodium thioglycollate	98.8±1.6
Sodium thiosulphate	101.6±2.4

Aqueous solutions of $CH_3{}^{203}HgCl$ (2 μg ml^{-1}, 0.05 μCi μg^{-1}) were incubated for 44 h at 37 °C in the presence of the above substances. The final concentration of the solid compounds was 10 mg ml^{-1}. The results are expressed as percentage of initial radioactivity remaining after 44 h. Figures shown are the means ± s.e. of five results.

amounts of H_2S (Table 1). The inability of the strong reducing agent sodium thioglycollate to volatilise CH_3HgCl suggests that the effect of H_2S on the alkylmercurial is not simply due to the reducing capacity of H_2S. Furthermore, H_2S did not reduce inorganic mercury to metallic mercury (unpublished observations of I.R.R.), which may be compared with the results of De Filippis and Pallaghy[8], who found that acetylene and ethylene could reduce mercuric salts to mercury metal. The volatilisation of CH_3HgCl cannot be ascribed to the acidic character of H_2S, even though the *p*H of the CH_3HgCl solution had decreased from 6.5 to 4.0 by the end of the incubation. Experiments in which CH_3HgCl was incubated in air or H_2S in the presence of buffers of *p*H 2.2–8.5, demonstrated that the *p*H of the solution had little influence over the rate of volatilisation by H_2S and, furthermore, in the absence of H_2S, virtually no radioactivity was lost at any of the *p*H values.

The rate of volatilisation increased linearly with concentration in the presence of H_2S, so that the percentage of CH_3HgCl volatilised was constant at all concentrations up to 50 μg ml^{-1}, the highest concentration tested. The rate of volatilisation was temperature dependent—at 37 °C about 90% of added alkylmercurial was lost from solution, whereas at 5 °C only 50% was lost.

We attempted to trap and identify the volatile product of the reaction between H_2S and $CH_3{}^{203}HgCl$ or $^{14}CH_3HgCl$ using a solution of sodium carbonate and disodium phosphate[9] (Table 2). Nearly all the evolved radioactivity, whether ^{203}Hg or ^{14}C, was absorbed by the carbonate–phosphate solution. In the absence of H_2S, little radioactivity was lost from solution and virtually none was found in the trap. In experiments described in Table 2 there was considerable variation in the amount of mercury volatilised, probably due to differences in initial H_2S concentration, or slight leakages from the desiccator seals. Consequently the differences between percentage loss of ^{203}Hg- or ^{14}C-labelled methylmercury have no significance, for the experiments were performed on separate occasions.

Table 2 Absorption of volatile mercury compound by carbonate–phosphate solution

Conditions	Radioactivity	% Radioactivity lost from flask	% Absorbed by CO_3/PO_4
H_2S	$CH_3{}^{203}HgCl$	23.3	22.9
	$^{14}CH_3HgCl$	16.3	14.4
Air	$CH_3{}^{203}HgCl$	1.3	0.0
	$^{14}CH_3HgCl$	0.05	0.06

A solution of $CH_3{}^{203}HgCl$ (2 μg ml^{-1}, 0.025 μCi μg^{-1}) or $^{14}CH_3HgCl$ (2 μg ml^{-1}, 0.01 μCi μg^{-1}) in a conical flask was placed in a desiccator which contained in its base a solution of sodium carbonate (10 g l^{-1}) and disodium phosphate (5 g l^{-1}). In two experiments the desiccator was flushed with H_2S for 2 min. After incubation for 3 d at 37 °C, samples were removed from the flask and from the absorber solution to assess the amount of radioactivity volatilised and absorbed.

The identity of the volatile product has not been fully elucidated. It does not seem to be metallic mercury because: (1) both ^{14}C- and ^{203}Hg-labelled volatile products were absorbed to the same extent by the carbonate–phosphate solution, which suggests that the C–Hg bond remains intact, and (2) metallic mercury vapour is not absorbed by the carbonate–phosphate solution which traps only organic mercury compounds[9].

When the radioactive contents of the carbonate–phosphate absorption solution were chromatographed on thin-layer plates of silica gel G-25 (Camlab, Cambridge), using two solvent systems, chloroform–*n*-hexane (9 : 1) (ref. 10), and *n*-hexane–acetone (7 : 3) (ref. 11), the radioactivity remained at the origin. But when the contents of the trap were extracted into toluene, all the radioactivity passed into the toluene phase and the radioactivity then migrated on the thin-layer plates at the same rate as a methylmercuric chloride standard, in the two solvent systems. Thin-layer chromatography of a sample of the radioactivity remaining in the flask revealed that it was CH_3HgCl and that no inorganic mercury was present. Thus, it seems likely that CH_3HgCl and H_2S react to produce a volatile sulphur derivative of methylmercury which, although stable in alkaline solutions, reverts to methylmercury on extraction into a polar solvent.

Fagerström and Jernelöv[12] have suggested that the presence of H_2S in sediments of lakes reduces the rate of methylation of mercury, due to the formation of mercuric sulphide, which is methylated much more slowly than mercuric chloride. The results reported here suggest that H_2S plays a further role in the turnover of mercury in an aquatic environment, that is, that any methylmercury discharged into a lake, or synthesised there by microbial action, may react with H_2S to form a volatile product which is released from the lake, thus making less methylmercury available for accumulation by fish and shellfish.

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Mixing in upper layer of a lake during heating cycle

Little is yet known about the structure of the upper 'mixing' layer of the ocean below the zone of direct surface wave influence. We report some measurements of the temperature field in the mixing layer of a freshwater lake during the heating cycle which demonstrate some of the processes which may be important. The temperature field is found to be isotropic at scales less than about 60 cm, but the effect of stratification becomes important at larger

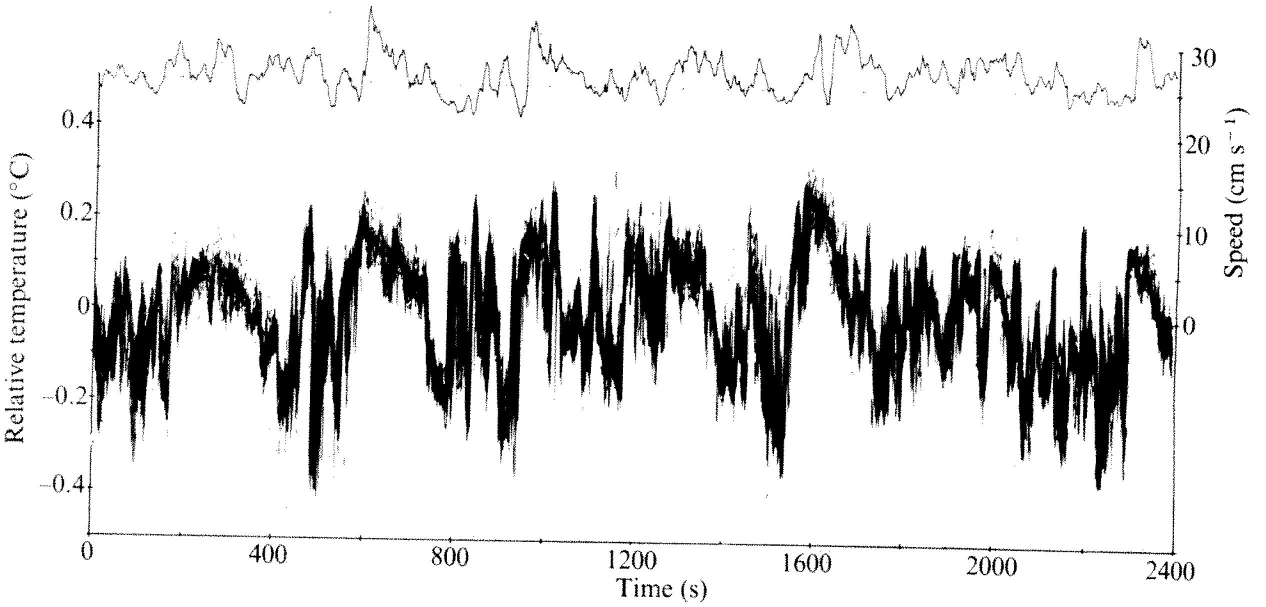


Fig. 1 Temperatures relative to their means and current recorded by the array of thermistors plotted against time. The record starts at 119GMT, July 14, 1976, when the centre of the array was at 6-m depth.

scales, reducing the vertical correlations in comparison with the horizontal. The spatial evolution of structures is dominated by the shear, as appropriate in the low Richardson number region. Examination of the stability of the mean velocity and density profiles shows that Kelvin–Helmholtz instability may be expected, leading to billows with wavelengths of about 60 m, and corresponding to fluctuation scales observed in the temperature records. Similar fluctuations have been seen in the nocturnal atmospheric boundary layer.

The measurements were made with a thermistor array in Loch Ness, 250 m from the north-east shore near Achnahannet, where the water depth is 170 m. The fetch at this site is over 20 km when south-west winds are blowing along the axis of the loch. The array consists of seven thermistors at 25-cm separation on a vertical rod, five at 45-cm separation in the direction of flow (orientation being maintained by a vane on the supporting tube) and three at 45- and 90-cm separation in the horizontal across the direction of flow. The sampling rate is 1 Hz and resolution 0.001 °C. The loch is freshwater, so density can be inferred from the temperature measurements. The array also carries a Savonius rotor current meter, a small vane and compasses to determine the flow direction.

Figure 1 shows the relative temperature values against time. The mean air temperature was about 1.8 °C in excess of the surface water temperature, and the wind speed was 10.6 m s^{-1} from the south-west. The dominant surface wavelength was estimated as 10 m, and whitecapping was frequent. There are fluctuations in temperature with a period of about 6 min and with a vertical scale which exceeds the array dimensions (the coherence between thermistors being high), as well as less coherent rapid fluctuations. The current speed is also shown in the figure and was directed in the wind direction with a variation of less than 20°.

We define the four-dimensional correlation function $C(x, y, z, \tau)$, the cross correlation between thermistors at relative position (x, y, z) at time lag τ by

$$C(x, y, z, \tau) = \frac{\overline{T(x, y, z, \tau + t) \cdot T(0, 0, 0, t)}}{(\overline{T^2(x, y, z, t)} \cdot \overline{T^2(0, 0, 0, t)})^{1/2}}$$

$T(x, y, z, t)$ is the variation of the temperature from its mean value, and is recorded at time t by a thermistor at a position (x, y, z), where x is directed north-east along the loch axis in the wind direction, y is across and z is vertically upwards. The overbars imply means taken with respect to time. Figure 2 shows sections of the constant C surfaces. For thermistor spacing <60 cm the $y = \tau = 0$ correlations in directions x and z are large and almost equal, but for larger scales the correlation in the vertical decreases

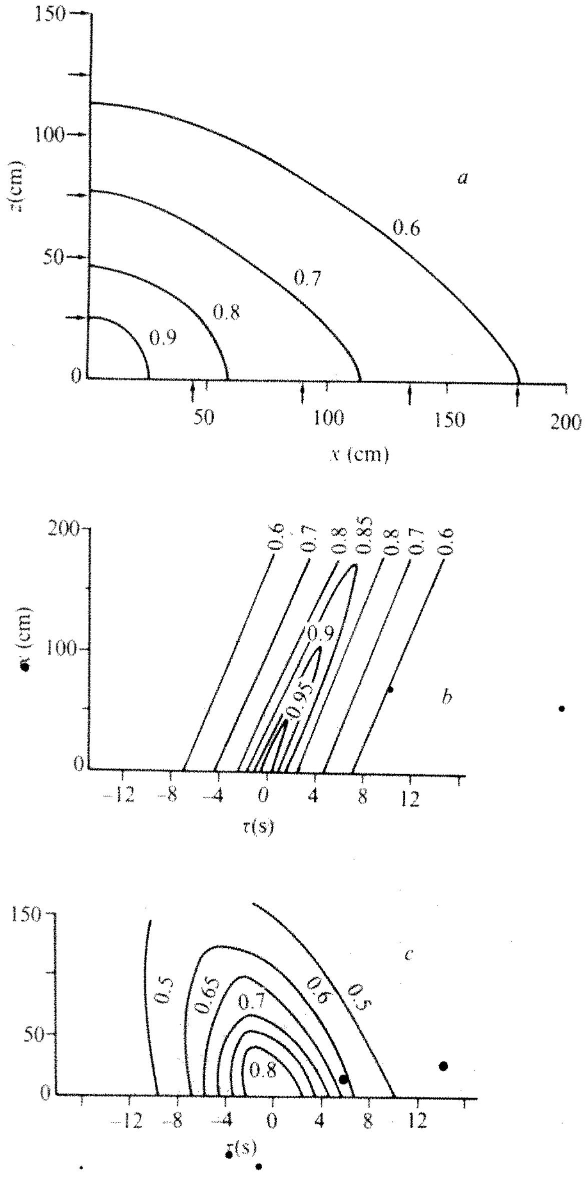


Fig. 2 Sections of the correlation function $C(x, y, z, \tau)$. *a*, Section $y = \tau = 0$. *b*, Section $y = z = 0$. *c*, Section $x = y = 0$.

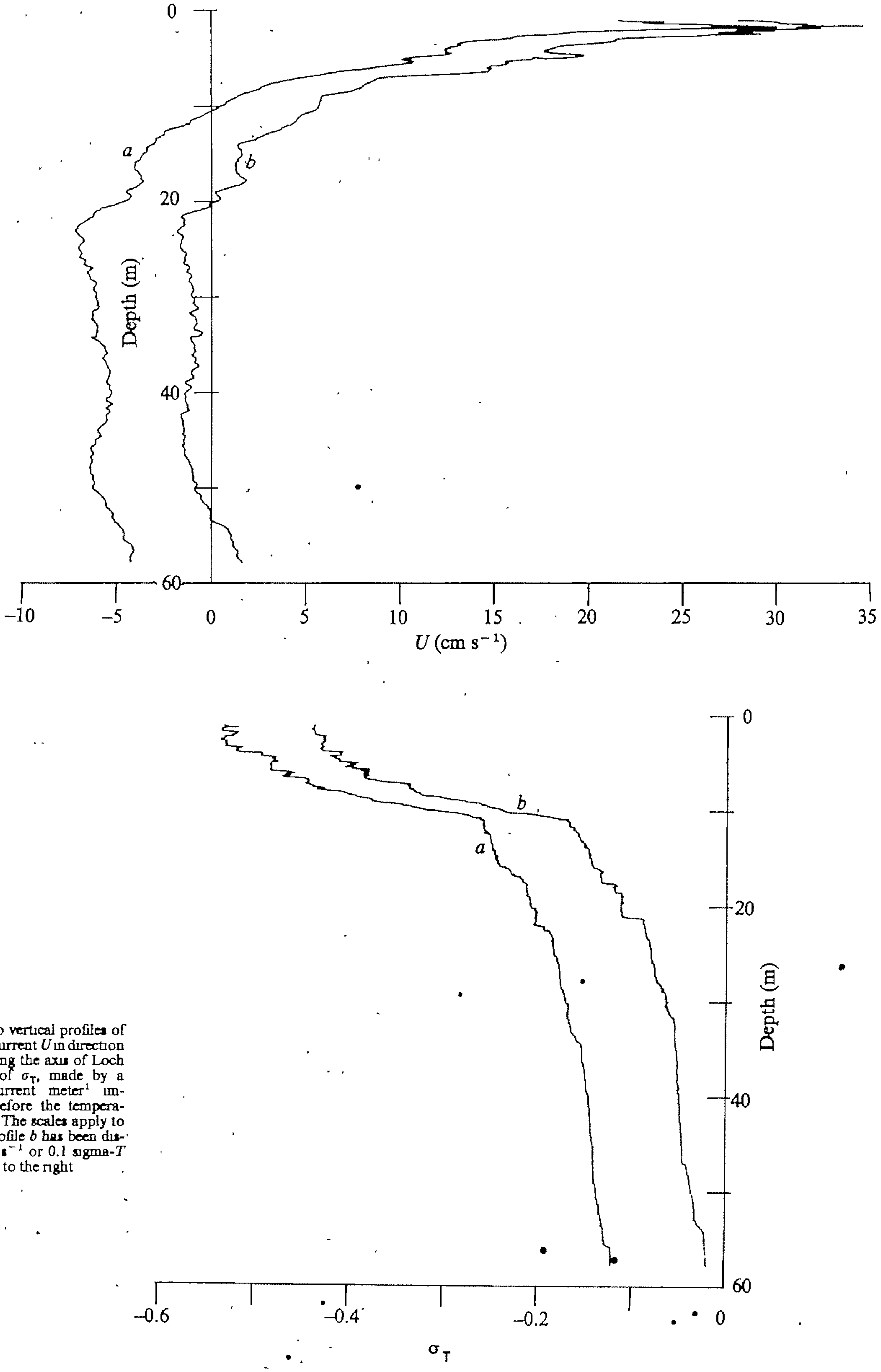


Fig. 3 Two vertical profiles of horizontal current U in direction 036 $\propto$ T along the axis of Loch Ness, and of σ_T, made by a profiling current meter[1] immediately before the temperature record The scales apply to profile *a* Profile *b* has been displaced 5 cm s^{-1} or 0.1 sigma-T units to the right

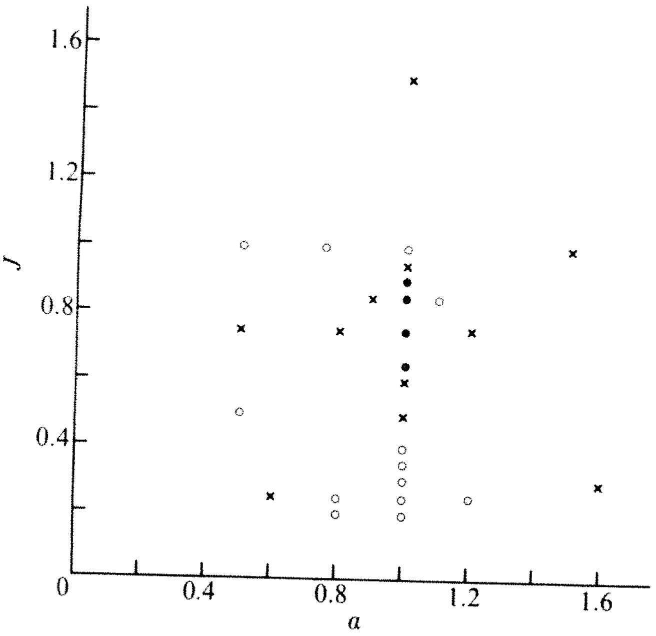
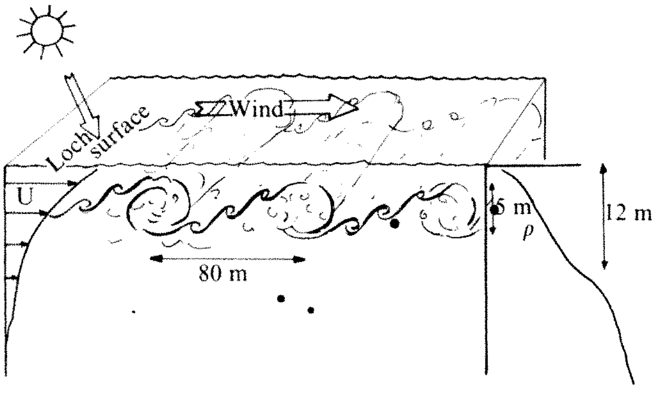


Fig. 4 The wave number, α, and Richardson number, J, plane, showing positions at which stable and unstable waves are found. The wavelength is $20\pi/\alpha$ (m) and the $J = 1$ corresponds to the observed mean profile. ×, stable waves; ○, waves which are unstable but have e-folding growth time exceeding 1 h; ●, unstable waves with shorter growth times.

more rapidly than in the horizontal, the ratio of horizontal to vertical scales at which $C = 0.6$ is reached being 1.59. The tilt of the constant C contours in the x–τ surface indicates the downstream advection of coherent structures through the array at a velocity which is, within the accuracy of the measurement, the same as the flow velocity. If the contours were all parallel, the correlation pattern would not change in time as it is advected past the array and the temperature field would be 'frozen' (the Taylor hypothesis). Figure 2*b* indicates that C decreases to 0.85 at $\tau = 7$ s as the pattern is advected and so, by linear extrapolation, $C = 0.6$ at about 19 s, and this is the evolution time scale of the temperature field. Figure 2*c* shows that the C contours in the z plane are also slightly skewed, indicating a downward propagation of coherent structures. No evidence of horizontal anisotropy was found in the analysis of the y correlations.

Figure 3 shows two profiles of current U and σ_t. The layers above 10 m are subject to a strong shear and contain frequent inversions in which the density decreases with depth. These have a r.m.s. scale of 76 cm, corresponding approximately to the scale at which the vertical coherence falls to about 0.7 and the anisotropy is first found between the vertical and horizontal structures. The Brunt–Väisälä period in this upper layer is about 600 s, so that the evolution scale of the thermal structures is not consistent with their being internal waves, which should have longer periods. The time scale of vertical shear, $(du/dz)^{-1}$, is, however, about 25 s and is comparable with the evolution scale. The apparent downward propagation of coherent thermal structures is consistent with the stretching of symmetrical structures in the mean flow. After the evolution time t, an initially vertical structure would have an inclination of $\tan^{-1}[(t\,du/dz)^{-1}]$ or 53° and this is consistent with the tilt and direction of tilt of the contours in Fig. 2*c*. During the process of distortion of such a symmetrical structure by shear the x correlations are unchanged, but the z correlations are reduced by a factor $1/(1 + t\,du/dz)$, giving a ratio of scales, $1 + t\,du/dz$, of 1.76. The estimates of tilt and ratio scales are affected by the choice of $C = 0.6$, but show reasonable agreement with observations, indicating a shear-dominated structure at the 1-m scale.

Fig. 5 A schematic diagram of the billows. The vertical scale is inferred from the mean vertical profiles and the magnitude of the temperature fluctuations measured by the array.



The equation which governs the growth of small disturbances to a parallel shear flow in a stably stratified fluid is the Taylor–Goldstein equation[2]. This has been solved numerically[3] for given wave numbers, α, and Richardson number, J, using values of U, the horizontal current along the loch axis, and N, the Brunt–Väisälä frequency, obtained from 1-m vertical averages of the profiles (Fig. 3), with linear interpolation between the lowest measured values and the bottom of the loch. Figure 4 shows the conclusions. The largest growth rate is found at $J = 0.9$, $\alpha = 1.0$, corresponding to a wave of length 63 m moving with a speed which increases its amplitude exponentially in a time of 22.4 min. This wave requires a Richardson number only 10% less than that observed, that is an increased mean shear of only 5%. These waves would have their orientation across the wind and current direction (Fig. 5), and may account for the longer period fluctuations in temperature seen in Fig. 1. Inversions occurred most frequently in the regions of large rapid fluctuations (e.g. near 100, 500 and 900 s), and these appear to be the locations of the overturning billows. Similar waves have been observed using radar in the stable nocturnal atmospheric boundary layer over land[4,5], and may provide an important vertical diffusion process in stable boundary layer flows.

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The age and extent of the Red Sea oceanic crust

THE evolution of the Red Sea depression and the extent within it of oceanic crust have been widely discussed[1–5]. It is generally accepted that the axial trough is floored by oceanic lithosphere formed at a constructive margin over the last 3–4 Myr by spreading at a rate of about 1.0 cm yr^{-1} per flank[2,6]. It is also clear from stratigraphic continuity and the trend of isofacial lines that the opening of the Red Sea started less than 45 Myr ago after a long period of tectonic and magmatic quiesence[7,8]. Two areas of contention exist. First, how much of the Red Sea on either side of the axial trough is underlain by oceanic crust, and second, when was this oceanic crust formed?

The sedimentary record shows that before the Middle Oligocene (32 Myr) a shallow sea extended southwards from the main 'Tethyan Ocean' on to the then contiguous Nubian–Arabian plate[8,9]. South of this sea was a low-lying flat or gently warped peneplaned land surface[8]. A drastic change occurred in the Red Sea at the beginning of the

Miocene (about 26 Myr). Evaporites began to be precipitated and these, by the end of the period (7 Myr), had attained thicknesses of 4–5 km. Also, coarse clastic sediments first appear as marginal intercalations within the evaporites[10,11]. It is obvious that from that time (27 Myr) uplift occurred periodically along the sides of the Red Sea, which was attaining something near its present shape and extent. So, uplift and the formation of the Red Sea linear depression seem to have started about 26 Myr ago. At about 30 Myr ago the eruption of 50,000 km^3 of trap basalts in Ethiopia and south-west Arabia started[4,7] although localised, minor activity had occurred before that time[12]. Both volcanism and uplift were active during the last 30 Myr although the main trap volcanism seems to have stopped by 24 Myr (ref. 13) before the major uplift took place[4]. Investigations in Afar[14–16] have established the alternating nature of vertical movements and volcanism; when there was volcanism there was little or no uplift and vice versa. In the Afar the processes started about 25 Myr ago[14] and have continued ever since with a cyclic periodicity of 2–4 Myr (ref. 16). Further investigation of the eastern Red Sea coast in southern Saudi Arabia[4] convincingly demonstrate the presence of a 22-Myr old linear volcanic zone in which dyke injection is related to crustal thinning and warping and the development of the Red Sea depression in that area.

Are the geophysical data compatible with this primarily geological scenario? There is no geological objection to the conclusion, based on seismic refraction[3] and magnetic anomaly[6,19] studies, that the Miocene evaporites which flank the axial trough are underlain by oceanic crust, although it could be argued that the sub-evaporite P-wave velocities of 6.6 km s^{-1} (ref. 3) are more characteristic of coarse-grained basic rocks of layer 3 than the finer-grained extrusives of layer 2. It is also geologically acceptable that the linear magnetic anomalies over the evaporites are oceanic strip anomalies whose amplitude has been reduced by the thick sedimentary overburden. Further, the near coast-to-coast fit of the Arabian plate to that of Nubia, based on plate tectonic theory, is in keeping with these data[5,20].

Girdler and Styles[6,19] compared the anomaly profiles from an evaporite-covered area on the west side of the axial trough between 15° and 16°N with the magnetic anomaly reversal timescale. They conclude, on the basis of the best anomaly profile match, that the oceanic crust beneath these evaporites was formed either 41–34 Myr ago with a spreading rate of 1.3 cm yr^{-1} per flank or between 29 and 24 Myr ago with a 2.3 cm yr^{-1} spreading rate. They preferred the older period as the profile match was better and the spreading rate more plausible. It is geologically difficult to accept the older period as this requires that crustal downwarp, transcontinental rifting and the formation of over three-quarters of the Red Sea oceanic crust occurred at a time when there is no geological evidence of major uplift, magmatism and crustal deformation anywhere in the region. The younger period (29–24 Myr) would indeed coincide with an episode of intense magmatic and tectonic activity, but it still leaves 20 Myr (24−4) in which it is supposed that no spreading occurred under the Red Sea, whereas magmatic activity and large scale vertical and horizontal movements occurred around the periphery.

The model that best fits the geological data is a Red Sea linear depression that formed in the late Oligocene–early Miocene (25–26 Myr), with sea-floor spreading continuing intermittently ever since. I attempt here to determine whether the magnetic data can be fitted into this model. The problems arise from the evaporites. They are, on all accounts, of Miocene age and are primarily composed of halite with minor anhydrite, shale and marl horizons. Invoking the law of superposition, if the evaporites are Miocene (26–27 Myr), then whatever is underneath must be pre-Miocene—older than 25 Myr. But must it? Evidence that this is not necessarily so comes from Girdler and Whitmarch[21] who describe DSDP sites 227 and 228 at the edge of the axial trough. Here, Miocene evaporites overlie oceanic crust confidently identified as aged less than 2.5 Myr on the correlation of the well-developed linear magnetic anomalies of the axial trough with the reversal time scale. They convincingly demonstrated that, as most of the evaporite is rock salt at temperatures above 100 °C at the base of the sequence, flow is not only possible but likely, and this is confirmed by petrographic studies (Shearman, personal communication). If Miocene evaporites have flowed over the recently formed axial trough oceanic crust, could not such flow have occurred throughout the development of the Red Sea? Recent volcanological[22] and ocean-floor studies[23] indicate that sea-floor spreading can be intermittent on a relatively short time-scale. It would be consistent with the Afar evidence and the cyclic occurrence of coarse clastic sediments within the marginal evaporites, if spreading along the Red Sea constructive margin had taken place intermittently over the last 25–30 Myr. If the spreading phase continued long enough and was fast enough, the overlying evaporites would part and an axial trough form, only to be obliterated by salt flow when spreading stopped. Conversely, if the constructive margin processes were slow and short-lived, then oceanic lithosphere would be produced beneath an evaporite cover; a locale far more likely to form coarse-grained basic rocks with a characteristic 6.6-km s^{-1} P-wave velocity[3].

There are two possible objections to this model. First, of the 105 km of post-Cretaceous sinistral movement along the Jordan rift[24], only 40–45 km can be proven to be of post-Lower Miocene age. Yet Freund *et al.*[24] claim that this 40–45 km of latest movement probably took place in the last 12–7 Myr and, accepting an average movement rate of between 0.4 and 0.6 cm yr^{-1} for the entire 105 km, they calculate that movement along the rift began 20–30 Myr ago (ref. 24, p. 124)—dates entirely in keeping with the proposals made here. Second, the ages of 36 and 30 Myr for a mildly metamorphosed argillite and basalt[25] at the bottom of two boreholes into the evaporites are quoted by Girdler and Styles[6,19] in support of the 41–34 Myr spreading phase. But, as well as the dubious validity of stratigraphically uncontrolled whole rock K–Ar age determinations, Lowell and Genik identify a continental setting for both the argillite and the basalt (ref. 25, p 249). It seems therefore that the proposal for a 41–34 Myr spreading phase must stand on magnetic anomaly matching alone. The veracity of this procedure for the Red Sea must be queried, for it produces results that conflict with the majority of other evidence.

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Andesitic pyroclastic flows from Colima Volcano

COLIMA (19°31′N, 103°37′W) is important as one of the most continuously active continental andesite volcanoes in North America. Various types of pyroclastic and lava eruptions have been reported over the last 400 yr (ref. 1). Here we describe the eruption of andesitic lava and associated pyroclastic flows during December 1975–April 1976. On the basis of our visits between January and June, 1976, we comment on the relationship of this to earlier eruptions of Colima.

Colima is a large composite volcano, now slightly over 4,000 m high, situated within a caldera[1] (5 km diameter) on the southern flank of the extinct Pleistocene volcano of Nevada de Colima (4,320 m, rising from a base level of 1,500 m). Colima forms part of the Mexican Volcanic Belt, which is associated with subduction of the oceanic Cocos plate below continental Mexico[2]. Eruptions of Colima have been noted since 1576 and to view the 1975–76 eruption in perspective we summarise the history during the present century.

In 1906, the volcano had a conical form,[1] with truncated summit and a small crater. A large explosive eruption in 1913 produced an extensive airfall deposit and 'nuée ardentes'[3–4], and left a much larger crater with a rim height of 3,960 m (ref. 4). Growth of a lava dome within the crater during 1913–35 was accompanied by mild explosive activity, and by 1935 the dome was 60 m below the crater rim. The volcano was quiescent until 1958, when the dome began to rise again so that its highest parts reached the level of the crater rim. This led to extrusion of a blocky andesite flow from the side of the dome down a prominent gully on the northern flank of the cone during 1961–62 (ref. 5; Fig. 1). Since 1962 the dome has grown slowly so that the volcano's height increased to an estimated 4,100 m by 1975, before the start of the latest eruption.

The eruption started on December 11–12 with mildly explosive activity, the extrusion of andesite lava in three small lobes to the north and two flows down the south-east flank of the cone (Fig. 1). Extrusion of the lobes was completed rapidly, probably within a few days, and they were static on January 3. On that date the east–south-east flow was moving very slowly but the most south-easterly flow was very active and continued to flow until studied in detail on January 14–15. In contrast with the lobes to the north of the cone, and the older 1961–62 flow, both south-easterly flows have prominent levées.

A variety of pyroclastic and rock deposits, below the north lobes and on the western side of the volcano below the summit dome, are associated with the early phase of the eruption. These include 'hot avalanche' deposits[6] erupted during lava or dome growth on a steep slope, colder avalanche deposits formed during cooling and disintegration of lava, and more slowly accreted rock-fall debris. During part of the early phase, juvenile andesite blocks up to 0.5 m³ were ejected explosively, falling up to 2 km from the cone.

Between January 3 and 15 there was strong fumarole activity from depressions in the summit dome (Fig. 1); occasional colder rock-falls, and active lava flow movement only on the most south-easterly flow. The lava flowed down the Barranca de la Arena (Fig. 1) and reached 2 km from the cone by January 14–15, when it was examined in detail at the point indicated on Fig. 1. At this time andesite lava was flowing down the barranca over a break of slope 50 m high. Flow of lava down the valley was accompanied by pyroclastic flows ranging from hot avalanches to a nuée ardente type of flow. The hot avalanches comprised large falls of hot (often flowing) rocks with total volumes up to 10 m³. The falls were from the steep, 40-m front face of the lava flow. The slope below this face was about 40°; this slope representing a steep section of the barranca, smeared with lava debris. The avalanches generally travelled up to 100 m down the valley and recurred every 4–5 min.

All gradations occurred between the hot avalanches and a pyroclastic flow resembling a small nuée ardente. The latter flow was initiated by the collapse of a substantial mass of hot (often glowing) blocks from the lava flow front. These were immediately engulfed in an expanding dusty cloud. After formation, the flow moved away from the lava front quietly (in great contrast to hot avalanches). The cloud expanded simultaneously in all directions away from the flow, eventually engulfing a considerable area (hundreds of m²) of the surrounding countryside and leaving a deposit of very fine brown dust. The cloud had cooled before engulfing the observation point, 200 m from the flow front.

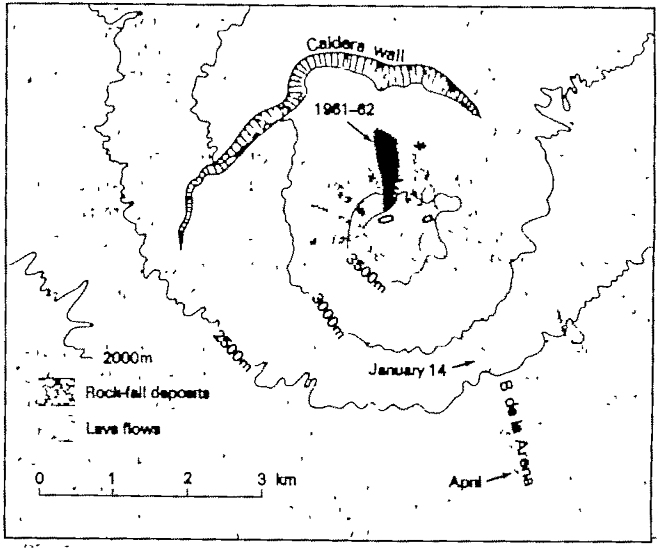

Fig. 1 Map of Colima volcano, showing lava and rock fall (including pyroclastic deposits) formed during the 1975–76 eruption. The outlined area labelled, 1961–62, extending to the north of the summit, is the lava flow erupted during these years. Solid lines on the summit enclose depressions in the lava dome which have abundant fumaroles.

We believe that the quietness of the pyroclastic flows is significant since it must indicate movement of blocks as a suspension within the flow. Arrest of the flow was accompanied by resumption of considerable noise, and blocks were deposited from the flow. The pyroclastic flows were also characterised by a narrow channel, a few m wide, in the underlying rock deposits. This was partly erosional in character but also reflected alignment of the deposited material. Such pyroclastic flows were formed every 7–8 min during the period, the earlier passage of flows down the same valley being indicated by destruction of trees at the top of the valley walls—the trees having been aligned down-valley. Between January 15 and April the lava flowed a further 1–2 km (Fig. 1) and movement ceased on June 20.

This type of pyroclastic flow has been reported from several volcanic areas, including Indonesia and central America (for example, Santiaguito, Guatemala[10]), and is similar to flows erupted at Merapi (Java) as a result of collapse of the side of a growing lava dome[7,8]. They have been termed 'Merapi-type' pyroclastic flows[8]. The pyroclastic flows were a common and characteristic part of the 1975–76 eruption of Colima, and we infer that they grade upwards in scale to larger nuée ardentes described from earlier eruptions of Colima and possibly formed at an earlier stage of the 1975–76 eruption. It seems likely that deposits from these eruptions would be difficult to identify with confidence in an older mixed succession of lavas and pyroclastics. We therefore suggest that such pyroclastic flows are more important than commonly supposed in the growth of composite andesite volcanoes. Although it is difficult to estimate, our study of Colima suggests that as much as a third of the erupted products of this volcano might be deposits from pyroclastic flows of the types described here.

Our studies of Colima were made during a visit sponsored by Earthwatch (Educational Expeditions International) and the Centre for Field Research (Belmont, Mass.). We thank the expedition participants for help in making the observations, E. Areola and L Flores for assistance in Cuidad Guzman and on Colima, and many friends in Mexico for help and encouragement.

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Detection of atmospheric infrasound by homing pigeons

ACOUSTIC waves of frequencies below 10 Hz are common in the atmosphere. They are generated by various sources, including wind, thunderstorms, weather fronts, magnetic storms, aurorae, ocean waves, earthquakes, and many of man's mechanical devices[1–3]. Many of these atmospheric oscillations are of high amplitudes (frequently well above 100 dB SPL at frequencies below 5 Hz, and above 120 dB below 1 Hz) but because they are outside the normal limits of human hearing they usually go unnoticed (although humans can detect extremely loud artificial infrasounds generated in test chambers, these sounds are louder than most natural infrasounds and verge on the threshold for pain)[4,5]. It has been suggested, however, that migrating and homing birds might make use of the cues provided by such infrasounds[6]. During our ongoing study of the sensory basis of avian orientation, we investigated this possibility. We report here that homing pigeons (*Columba livia*) are sensitive to infrasounds at reasonable amplitudes.

Our testing procedure, using a conditioned cardiac response (increased rate of beating), was very similar to one used to test for sensitivity to barometric pressure changes[7]. The pigeon was fitted with two ECG surface electrodes and two shock electrodes (implanted under the skin of the breast), restrained by a leather harness, and placed in a sound-insulated conditioning chamber 30×44.5×30 cm high (the chamber was described more fully in the barometric pressure study[7]). Fresh air for respiration was supplied at 5 l min^{-1} through a system of pressure and vacuum pumps; however, this input was cut off and the chamber completely sealed for short periods while tests at very low frequencies were run (this reduced the background noise in the chamber by about 20 dB).

Two methods of producing acoustic pressure oscillations were used. In some of the early tests, we used a motor-driven piston of approximately 1 cm diameter, with an adjustable stroke to produce changes in amplitude. This apparatus was modified from a Phipps and Bird small animal respiration pump, model 70-878-01. The pressure output was a sine wave and the frequency was varied by changing the motor speed. The piston was always stopped at the mid-point in the cycle, insuring that the next stimulus would start at zero amplitude. In later experiments, the pressure variations were produced by a loudspeaker driver (Atlas sound PD-60-T) connected to the chamber with tubing so as to act on a closed volume of air. In such a closed system, the loudspeaker driver was not limited to the normal free-air frequency response, but instead could produce low-frequency pressure changes in the chamber well below 0 1 Hz. The loudspeaker driver was powered by a Hewlett-Packard sine wave generator, model 200 C. With both methods of producing pressure changes, the sounds were piped through approximately 2 m of plastic tubing 1 cm in diameter and passed through a mechanical lowpass filter before entering the chamber. The filter served to reduce high-frequency sounds in the audible portion of the spectrum. The lowpass filter was constructed of a cylinder 5 cm in diameter and 15 cm long, coaxial with the 1 cm tubing and packed with glass wool. The frequency characteristics of the filter will be described in detail elsewhere. The rise and fall times of the stimulus envelope were controlled to virtually eliminate high-frequency transients.

Stimulus presentations were at random intervals. Before each presentation, the heart rate of the pigeon was monitored for 5 s; this was followed by a 10 s stimulus, which was in turn followed immediately by a shock (1 mA for 350 ms from a 60 Hz a c. constant-current source). After a few trials, a conditioned response developed, and the heart rate increased each time the bird detected the stimulus, before the shock. For a positive response to be counted, the heart rate during the 10 s stimulus period must have exceeded by at least 12 beats per min the highest rate recorded during the preceding 5-s baseline period. Typical baseline rates were approximately 140 beats per min, and accelerations in response to stimuli ranged from 30 to 60 beats per min. Instantaneous heart rate was monitored with a Grass Instrument 7P4 tachograph, with a linear voltage output proportional to heart rate, measured on a beat-to-beat basis. A permanent record of the heart-beat rate was made on a Sanborn 350 chart-recorder.

Our experiments demonstrate that homing pigeons can detect sound energy below 10 Hz at amplitudes well within the range of naturally occurring sounds at these frequencies. Indeed, they can detect frequencies below 1 Hz. At 113.8 dB r.m.s. SPL (1 mm H$_2$O), most birds responded to stimuli of 1.67 Hz and 0.8 Hz at a much higher rate than background

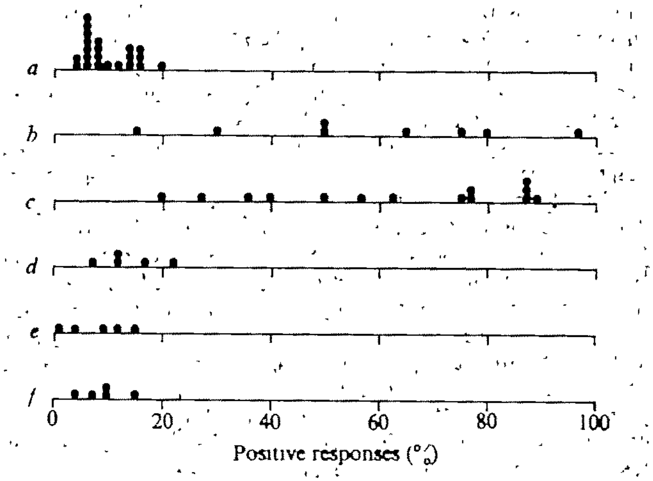

Fig. 1 Percentage of positive responses of individual homing pigeons to infrasound stimuli (40 presentations each). *a*, Chance 'positive' response levels of 22 birds when no stimulus was provided; this is the background level against which responses in other test situations should be evaluated. *b*, Responses of eight pigeons to sounds of 1.67 Hz. *c*, Responses of 14 pigeons to sounds of 0.8 Hz. *d*, Responses of five pigeons to sounds of 1.67 Hz when the tubing between the sound generator and the test chamber was closed by pinch clamps. *e*, Responses to sounds of 1.67 Hz by five pigeons whose cochleas and lagenas had been removed. *f*, Responses to sounds of 1.67 Hz by five pigeons whose columellas had been bisected. All sound amplitudes were 113.8 dB rms SPL.

(compare Fig. 1*b* and *c* with 1*a*). The response could be eliminated if two pinch clamps were placed on the tubing between the sound generator and the test chamber (Fig. 1*d*).

From preliminary experiments, we estimate the threshold (50% response) to be approximately 80 dB at 1.5 Hz, 60 dB at 5 Hz, and 50 dB at 10 Hz. These compare with published values for human thresholds of 132 dB at 1.5 Hz, 115 dB at 5 Hz and 104 dB at 10 Hz (ref. 5). The ambient noise levels inside the insulated chamber were 70 dB at 1.5 Hz (band width (bw) 10% of centre frequency), 50 dB at 5 Hz (bw 1%) and 47 dB at 10 Hz (bw 1%); inasmuch as the 50% threshold values were only slightly above these noise levels, it seems possible that the pigeons would be even more sensitive to signals of these frequencies if tested in a quieter chamber. (A more detailed study of threshold response values, down to 0.1 Hz, is in progress.)

To determine the location of the receptors responsible for infrasound detection, we tested five pigeons from which the cochleas (and lagenas) had been surgically removed, and also five pigeons with intact cochleas but with both columellas bisected so that there was no direct conduction of sound between the tympanum and the oval window. Figures 1*e* and *f* show that neither of these groups of birds respond to an infrasound stimulus of 1.67 Hz at 113.8 dB; the response levels for both groups were indistinguishable from those of normal birds in the absence of a stimulus. These results suggest that the receptors for infrasound are in the inner ear, but it is not clear which organ is responsible because removal of the cochleas and lagenas unavoidably damages the remaining portions of the labyrinth, one part of which has recently been shown to function (at least in frogs) as an auditory receptor[8].

Unlike sounds easily audible to humans, atmospheric infrasounds can travel huge distances—often thousands of miles—without much attenuation (which is proportional to the square of the frequency). Hence such sounds could potentially provide birds with much useful information. For example, the ability of birds to sense approaching weather fronts might be aided by detection of infrasounds funnelled to earth by those fronts or by detection of the infrasound components of thunder associated with storms that are still hundreds of miles away. Localisation of several remote infrasound sources could serve as a distant landmark system for triangulation of the home site and of the location of a release site. Properly utilised, infrasound information could thus assist in almost every aspect of avian navigation, in both homing and migration.

Whether one or more of these potential uses is actually realised is not known. There are several reasons why birds might have difficulty extracting meaningful information from infrasounds. For example, they would need to distinguish between spatially coherent infrasounds and so-called pseudosounds, which are very local pressure oscillations generated by winds and atmospheric turbulence. Further, the usual binaural comparison methods of localising sounds would be ineffective where the wavelengths are so very long (approximately 300 m for a sound of 1 Hz). Neither of these problems should be insurmountable for a bird in flight. Thus a flying bird might be able to reconstruct the spatial patterns of atmospheric infrasounds and thereby separate them from local pseudosounds. Quine and Konishi[9] have shown that, in the usual audible range, owls can distinguish frequencies that differ by as little as 0.5–0.7%; if such discrimination holds for pigeons in the infrasound range, then doppler shifts in frequency, which for a pigeon flying at 20 m s^{-1} toward or away from the sound source would be as high as 14%, could provide ample information for localisation. Only further research can reveal whether or not this is mere speculation.

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Genetic influences and infantile autism

IN his original description of infantile autism, Kanner suggested an "inborn defect", because symptoms were often present from early infancy. Despite the rarity of a family history of autism and lack of a known increase in parental consanguinity, there are two reasons for suspecting hereditary influences: the 2% rate of autism in siblings is 50 times that of the general population[1], and a family history of speech delay is found in about a quarter of families[2]. Reports of single pairs of twins with autism have not added much to our knowledge of genetic effects because of a bias toward reporting monozygotic (MZ) concordant pairs and because few reports contain both adequate clinical descriptions and evidence of zygosity[1]. We therefore undertook a study of a systematically collected sample of 21 pairs of same-sexed twins, one or both of whom had autism as diagnosed by the criteria of Kanner[3] and Rutter[4]. The results reported here indicate the importance of hereditary influences in the aetiology of autism.

The names of twins were collected from consultants, schools and units for autistic children, the National Society for Autistic Children and two hospital twin registers. Apart from two pairs in which dermatoglyphics were used, zygosity was determined by blood grouping in all cases not markedly different in physical appearance. Case notes of the 33 pairs thus obtained were reviewed and eight pairs were rejected on diagnostic grounds. The remaining 25 pairs and their families were extensively interviewed, tested and examined at home or in hospital. Obstetric records and case notes were also studied. On the basis of all information, a further four pairs were excluded as not meeting diagnostic criteria.

The adequacy of our sampling is shown by the MZ: DZ ratio (11 : 10) which was about that expected for same-sexed twin pairs surviving the first year (6 : 7); and by the number of autistic twin pairs found. In the English twin population of school age who would meet our diagnostic criteria, between 19 and 27 autistic children would be expected and 20 were found. Our sampling of children over school age was less complete, but not likely to be biased as to zygosity or concordance since most cases came from twin registers.

The diagnoses were made from case summaries, numbered randomly to prevent sorting by pair, and from which name, exact age, and zygosity had been deleted.

At least one member of 21 same-sexed pairs of twins met the diagnostic criteria for autism. In all, the 11 MZ and 10 DZ pairs gave rise to 25 autistic children. Apart from being twins, these were similar to other reported series of autistic children with respect to sex ratio (3.4 : 1), social class (57% from I and II), and IQ (about 1/2 severely retarded but 1/4 with normal non-verbal IQ). Affective disorder was described in four families, but schizophrenia did not occur. One sibling suffered from autism (a rate of 2.8%). Delayed speech was reported in three families (Table 1).

Four of the 11 MZ pairs but none of the 10 DZ pairs were concordant for autism ($P=0.055$). All concordant pairs were male. Because autism is a rare condition (about 4 per 10,000 children), the MZ concordance rate is equivalent to a very high correlation in liability (over 0.9) using the multifactorial model[8,9].

If, using this model, it is hypothesised that autism reflects some form of continuously distributed, abnormal characteristic, the question arises whether the non-autistic co-twins showed any other abnormality. We found that very few did in the DZ pairs but in most MZ pairs the co-twins showed some form of cognitive disorder (see Tables 1 and 2).

Table 1 Pair-wise concordance by zygosity

	MZ pairs ($n = 11$)	DZ pairs ($n = 10$)	MZ–DZ difference (exact test)
Concordance for autism	36%	0%	$P = 0.055$
Concordance for cognitive disorder (including autism)	82%	10%	$P = 0.0015$

Thus, in addition to the autistic twins, 6 non-autistic co-twins (5 MZ and 1 DZ) showed a cognitive abnormality as defined by the criteria of no phrase speech until 3 yr (three cases), IQ 70 or below (two cases), severely abnormal articulation after 5 yr (two cases), or scholastic difficulties requiring special schooling (two cases). Four non-autistic twins showed only one of the above features and none were severely handicapped. All the autistic twins met at least two of the criteria. Thus, 9 (82%) of the 11 MZ pairs were concordant for cognitive disorder/autism compared with only 1 (10%) of the DZ pairs ($P=0.0015$). The findings strengthen the suggestion of genetic determination and indicate that what is inherited is a form of cognitive abnormality which includes but is not restricted to autism.

Table 2 Summary of findings for MZ pairs

	First-born twin	Second-born twin
1	Autism + severe MR	Autism + severe MR
2	Autism + mild MR	Autism
3	Autism + severe MR	Autism + severe MR
4	Autism	Autism + mild MR
5	Mild MR	Autism + severe MR
6	Autism	Educational difficulties (ESN school)
7	Severe articulation defect	Autism
8	Autism + severe MR	Language disorder
9	Mild MR	Autism + severe MR
10	Autism + mild MR	Normal
11	Normal	Autism

MR, Mental retardation.
ESN, Educationally subnormal.

Twins have a raised rate of perinatal difficulties leading to brain injury and it was necessary to check that the concordance was not an artefact of these. We found that it was not. In none of the four pairs concordant for autism did both twins have a history of brain injury. Furthermore, no twin with cognitive disorder in the absence of autism showed evidence of perinatal brain injury (Table 3).

Brain injury was, however, relevant to discordance

Table 3 Summary of biological hazards in discordant pairs

Hazard	Autistic twin	Non-autistic twin
	MZ pairs	
Definite	Multiple congenital anomalies	—
	Neonatal convulsions	—
Possible	Severe febrile illness	—
	Pathologically narrow cord	—
None	—	—
	—	—
	—	—
	DZ pairs	
Definite	Apnoea	—
	Delay second birth	—
	Delay second birth	—
	Delay second birth	—
Possible or Difference in severity	Severe haemolytic disease + apnoea	Delay second birth
	Severe haemolytic disease + apnoea	Mild haemolytic disease
	Birth weight 1 and ¼ pounds lower	—
	Birth weight 1 and ¼ pounds lower	—
None	—	—
	—	—

regarding autism. We identified five features known to be associated with brain injury: kernicterus, perinatal apnoea of more than 6 min, neonatal convulsions, multiple congenital anomolies, and a second birth delayed by at least 30 min. In 6 of the 17 pairs discordant for autism, the autistic child but not the non-autistic co-twin had experienced one or more of these biological hazards in the perinatal period.

A wider definition of biological hazard was then applied to the 11 cases not differentiated on the stricter criteria. Using the wider definition (lower birth weight by 1 pound or more, pathological narrow umbilical cord, a more severe kernicterus with apnoea and a severe febrile illness—possibly encephalitis–just before onset), a further six discordant pairs were differentiated. Thus, in 12 of 17 pairs discordant for autism, the autistic member probably or possibly suffered a brain injury, whereas in none of the discordant pairs did this occur only in the non-autistic member.

To summarise the results: the markedly higher rate of concordance in MZ, compared with DZ, pairs for both autism and cognitive abnormalities indicates the importance of genetic factors in the aetiology of autism. Indeed, the concordance rates in conjunction with the very low prevalence of autism in the general population point to a strong genetic determination. Nevertheless, the finding of an association between biological hazards in the perinatal period and autism also demonstrates the important role of environmental influences for the causation of autism (but not cognitive disorder).

In some cases, genetic factors seem to be both necessary and sufficient causes. Among the eight autistic children in the four concordant MZ pairs, biological hazards (even using the broader criteria) occurred in only one child. He was more severely affected than his twin. On the other hand, brain injury alone seems to be a sufficient cause in some cases. In one of the two completely discordant MZ pairs, the autistic twin had experienced neonatal convulsions, whereas the non-injured co-twin was normal in every way. The importance of brain damage is also indicated by the finding in other studies of autism in children with congenital rubella or infantile spasms.

Finally, some cases seem to result from the combination of an inherited cognitive defect plus brain damage. In three of the five MZ pairs discordant for autism but concordant for cognitive deficit, the autistic child had experienced a biological hazard whereas the cognitively impaired non-autistic co-twin had not.

Determining the mode of inheritance is difficult since autistic children rarely reproduce. It would be helpful to know what happens to the offspring of the non-autistic twins with cognitive impairment, but no such information is available.

In summary, we conclude that this systematic study of 21 same-sexed twin pairs in which at least one twin showed the syndrome of infantile autism, indicates the importance of a genetic factor in the aetiology of autism. It also indicates the importance of brain injuries, especially during the perinatal period, which may operate either by themselves or in combination with a genetic predisposition involving language. Both the mode of inheritance and exactly what is inherited remain uncertain.

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Anti-fouling role of antibiotics produced by marine algae and bryozoans

A GRADIENT of antibacterial activity along fronds of the large brown seaweed *Laminaria*[1] runs opposite to gradients of density of bryozoans and spirorbid tube worms settled on the fronds[2,3]. Bryozoan colonies attached to a *Laminaria* frond grow towards the younger region[4], where antibiotic production is lowest. It has been suggested that tannins produced by branch tips of *Sargassum* have an anti-fouling role[5], but we have seen no other clear evidence of such an effect, although many marine plants and animals remain remarkably free from encrustation by other organisms[6]. We have now found further evidence in several examples of differential settlement by spirorbid worms on algae and by a bryozoan on another species of bryozoan.

We had noticed that holdfast branches of *Laminaria digitata* (Huds.) Lamour on Pembrokeshire and Plymouth shores bear populations of *Spirorbis inornatus* L'Hardy and Quièvreux, always attached to the sheltered inner sides. The same spirorbid settles abundantly on *Himanthalia elongata* (L.) S. F. Gray, but only on the undersides of the button-like expansions which protect the attachment points of the alga. In laboratory experiments, which followed the usual procedures[7] and will be reported in detail elsewhere, we found that settling *Spirorbis* larvae invariably select those parts of the two algae, irrespective of the contours of the surfaces and of their orientation to light and gravity. We tested these two sites for differential production of antibiotics.

Disks 5 mm in diameter cut from the surfaces were tested by standard procedures[8] against *Staphylococcus aureus*, strain NCIB8244. This grew readily or formed indistinct clouded zones around surfaces normally shaded and colonised abundantly by epibionts (Fig. 1*a* and *c*), but left

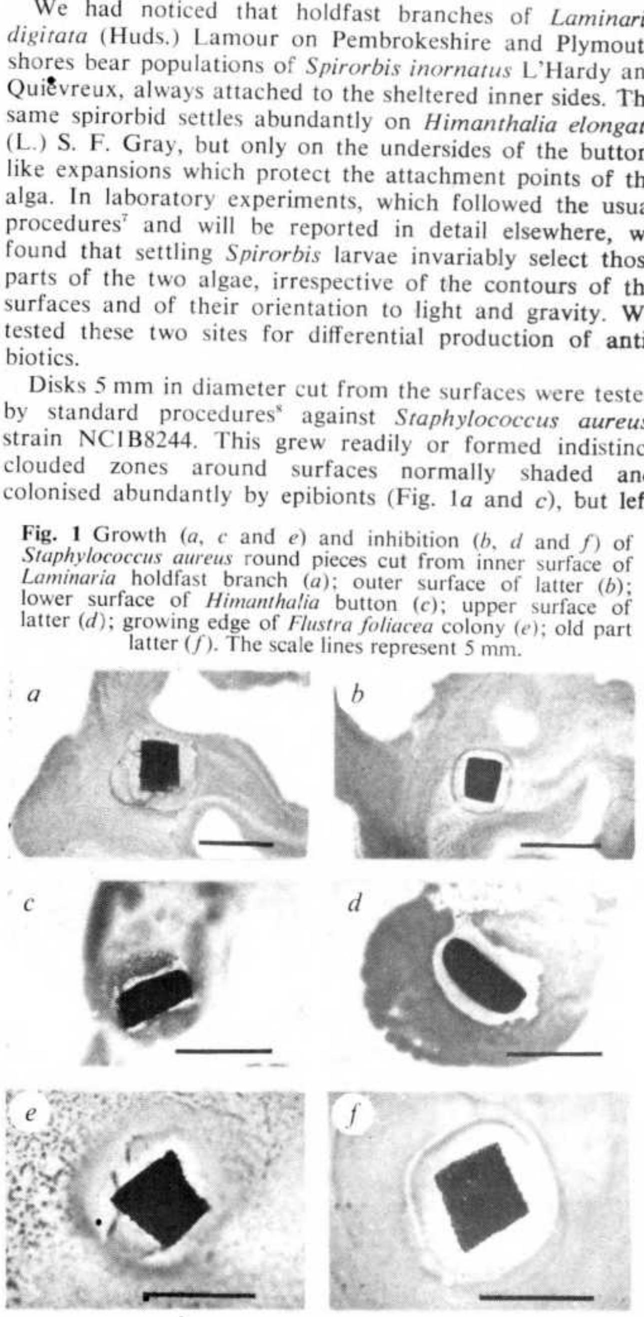

Fig. 1 Growth (*a*, *c* and *e*) and inhibition (*b*, *d* and *f*) of *Staphylococcus aureus* round pieces cut from inner surface of *Laminaria* holdfast branch (*a*); outer surface of latter (*b*); lower surface of *Himanthalia* button (*c*); upper surface of latter (*d*); growing edge of *Flustra foliacea* colony (*e*); old part latter (*f*). The scale lines represent 5 mm.

clearer and wider zones of inhibition around those normally exposed to light and unfavourable for the settlement of *Spirorbis* larvae (Fig. 1*b* and *d*).

We similarly tested the frondose bryozoan *Flustra foliacea* (L.), which is readily colonised by the bryozoan *Scrupocellaria reptans* (L.), with the initial settlement of larvae concentrated around the distal growing edge of the colony[4,9]. We found that region to be virtually devoid of antibacterial activity, whereas clear zones of inhibition usually appeared round pieces from older parts of the fronds (Fig. 1, compare *e* and *f*). The differences between pairs in each of the three experiments illustrated in Fig. 1 were all highly significant (Table 1).

Table 1 Mean widths of inhibition zones around 5-mm disks

Species	Region	No. of replicates	Mean width (mm) ± s.e.	*P*
Laminaria	Holdfast inner side	16	0.81 ± 0.13	< 0.01
Laminaria	Holdfast outer side	16	1.59 ± 0.15	
Himanthalia	Button lower side	24	0.28 ± 0.09	< 0.01
Himanthalia	Button upper side	24	1.00 ± 0.10	
Flustra	Growing edge	24	0.35 ± 0.11	< 0.01
Flustra	Old part of frond	24	1.35 ± 0.35	

Pieces cut from older parts of *Flustra* fronds, kept in a stoppered tube, emitted for several days a characteristic smell of lemons, whereas similar pieces from growing edges had a different smell and soon decomposed. This deserves further study, considering the insect-repellent reputation of citronellal, the apparent distaste for citronella grass shown by grazing buffalo[10] and the antiseptic properties of some monoterpene derivatives. The suggestion that tannins are the active agents in algae is supported by the finding that polyphenol production in *Laminaria* holdfasts seems to be increased at higher illuminations[11].

The settlement behaviour and growth orientation noted would confer advantages on the epibionts, ensuring a longer lifespan on *Laminaria* fronds, less competition for space on newly formed surfaces and greater protection from sand inside holdfasts or under *Himanthalia* buttons. Populations of *Spirorbis inornatus* are to some extent adapted to secure such advantages, for most larvae from parents on *Laminaria* settle on *Laminaria* rather than on *Himanthalia* (our unpublished results) whereas most from *Himanthalia* parents show the reverse preferences. But larval settlement on the insides rather than the outsides of *Laminaria* holdfasts, and on the lower rather than the upper sides of *Himanthalia* buttons, is not invariably the result of natural selection for avoidance of abrasion by sand, for similar behaviour is shown by larvae derived from a population of *Spirorbis spirorbis* L. at Swansea, which is confined to *Fucus* in pools 30 m from the nearest *Laminaria* (which is never colonised by *Spirorbis* in that locality) and 30 miles from the nearest *Himanthalia*. There could have been no recent evolutionary adaptation of that population to *Laminaria* or *Himanthalia*. The algae, on the other hand, presumably benefit from keeping their exposed surfaces clear for photosynthesis and there seems to be little need to discourage settlement on the sheltered surfaces inside holdfasts and under *Himanthalia* buttons. The antibiotics of *Flustra and Laminaria* seem to discourage only larvae, and are not obviously deleterious to older epibionts, which grow readily, no doubt protected by their greater size. The settlement on areas of new growth is not due to inability of growing regions to produce antibiotics, for several species of algae show maximum antimicrobial activity in regions which grow fastest[1,5]. In those species the growth regions are distal, suggesting that the antibiotics are involved in protection against epiphytes and macrograzers, as well as against the pathogenic invasions which would tend to follow damage by abrasion.

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Cell lineage analysis of germ cells of *Drosophila melanogaster*

THE initial events of differentiation of genetically identical nuclei within the eggs of higher organisms are generally believed to be triggered by a heterogeneous distribution of substances within the egg[1,2]. One of the clearest examples of such differential distribution of determinative substances concerns the posterior polar plasm of some insects. Morphological observations suggest that all cleavage nuclei in such insects have identical developmental potential, and that only the few nuclei that chance to migrate to the posterior polar region and thus to include a sufficient quantity of the morphologically distinguishable polar plasm, acquire the capacity to give rise to the germ line[3]. Various experimental approaches[4–8] have provided very strong, although not compelling [9] evidence for such a causal relationship between the polar plasm and germ line differentiation. Genetic fate mapping experiments described here strongly suggest that the germ cells of *Drosophila melanogaster* originate from the posterior-most region of the blastoderm, and they unequivocally rule out the possibility that the germ cells originate from a mid-dorsal position[10]. The posterior-most location of the germ cells supports, or at least is consistent with, the notion that the polar plasm determines whether or not a given cleavage nucleus will acquire the capacity to differentiate into a germ cell.

A priori, two possibilities can be distinguished. The first, as outlined above, states that "any nucleus may become a polar nucleus if it happens to move into the region of the posterior polar plasm, regardless of cell lineage" (ref. 3). The second assumes that the prospective germ cells are set aside during early cleavage before their interaction with the posterior polar plasm or with any other cytoplasmic region. One critical test between these two alternatives involves genetic determination of cell lineage relationships of the primordial germinal nuclei: only the first requires that they are mapped to the area of the blastoderm that contains the posterior polar plasm. The reason for this prediction is apparent, for example, from the observation that one ancestral nucleus in the posterior pole region can give rise to one daughter cell which contains the visible polar granules and one which does not[3], and hence that presumably only the former is likely to contribute to the germ line.

The technique of genetic fate mapping[11,12] provides one approach by which cell lineage relationships of various anatomical regions can be determined[8]. Using this technique, Falk *et al.*[10] located the gonads on the genetic fate map of *D. melanogaster* "about half way along the blastoderm near the mid-ventral line". They suggested therefore that "the assumption that the site at which a nucleus lands in the periplasm decides its fate has to be modified, at least for nuclei destined to give the gonads" (ref. 10).

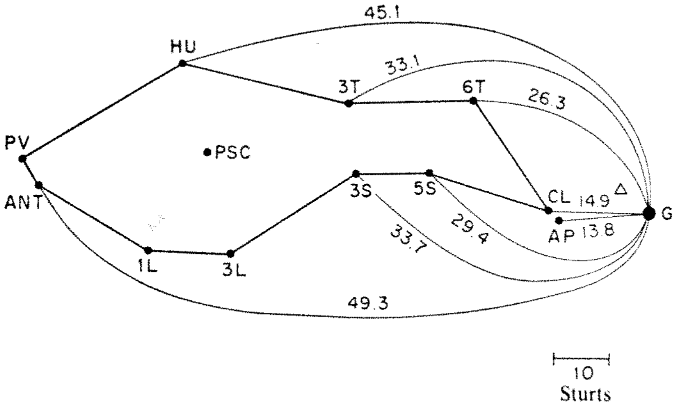

Fig. 1 Genetic fate map of the right half of *D. melanogaster* blastoderm, with special reference to the position of the germ cells. Because it was not possible to rule out a *priori* a bilateral symmetrical origin of the germ cells, data from germinally mosaic males were not included in the calculations of distances between the germ cells and various cuticular landmarks. But the general outline of the map, and, in particular, the posterior position of the germ cells, is preserved when the data from germinally mosaic males are also included or when the map distances are computed according to the suggested algebraic correction[19,22] for systems in which the mean proportion of mutant tissue is significantly different from 0.5. The open triangle represents the position of the germ cells triangulated on a map derived from 105 mosaic females and then superimposed on the map of 215 phenotypically mosaic males shown here. ANT, antenna; AP, anal plate; CL, clasper; GC, germ cells; HU, humeral bristles; PSC, posterior scutellar bristle; PV, post-vertical bristle; 1L, first leg; 3S, third hemisternite; 3T, third hemitergite.

Both the observations that some pole cells in *D. melanogaster* give rise to somatic cells rather than primordial germ cells[13], and the observation that in the wasp *Pimpla turionellae*, although characteristic pole cell formation and migration occur, germ cells are found in the absence of pole cells[14,15], seem to be compatible with this suggestion. But two other attempts[16,17] have been made to determine the developmental origin of the gonads by means of genetic fate mapping, and these gave a posterior position compatible with the hupothesis that the site at which a nucleus lands in the cytoplasm decides its fate. These inconsistent results are surprising in view of the reproducible interrelationships between cuticular landmarks that are reflected by many independently derived maps[11,12,16-23] and in view of the strong evidence for the validity of the fate mapping procedure[12,21,24].

All three investigations mapped whole gonads either at the larval stage[10,17] or at the adult stage[16]. It is well established that the gonads of *Drosophila* derive from two developmentally unrelated components: the germ cells which give rise to the germ line and mesodermal cells which give rise to the gonadal sheath and interstitial cells[8,25]. The nature of the interaction between these two components is unclear[13,25]. In addition, the attachment of testes in adult gynandromorphs to the developmentally distinct female oviducts causes degeneration of the testes[26]. These theoretical considerations, coupled with the technical difficulties encountered in fate mapping of internal organs and the need to use a functional, rather than morphological criterion for the germ nuclei, make it very difficult to resolve these contradictory results and their bearing on the hypothesis that the posterior polar plasm contains germ cell determinants. A clearcut resolution of this problem is therefore likely to emerge only from functional mapping of the germinal nuclei themselves, irrespective of other elements of the reproductive system[8]. The experiments described here constitute the first clearcut genetic evidence for the developmental origin of the germ cells, and they show that the germ cells of *Drosophila* do indeed originate from the general area that has been traditionally assigned to them by cytologists—the posterior tip of the egg.

Males of the constitution *y*–*y*$^+$ *Y*; *pal*–*pal* were crossed to XY, *y B* females[20,27]. The sons from this cross were screened for *pal*-induced somatic mosaicism for the extra Y chromosome; this can be detected by the appearance of yellow and non-yellow cuticular patches in the same male. The frequency of mosaicism among the F_1 males has not been calculated directly, but ranged approximately from 0.001 to 0.002. On average, each cuticular region was yellow in 40% of the mosaic males, non-yellow in 55% and mixed in the remaining 5%. The mosaic distribution of the entire cuticle of each rare mosaic male was recorded. Subsequently, each mosaic male was crossed to five to nine *yellow* females. These *yellow* females were kept in a stock in which all males carried the B^sY chromosome and thus any non-virgin yellow female could be detected from a phenotypic examination of her sons. The constitution of the germ cells of each 0–14-d old mosaic male was inferred from the phenotype of his sons: the germ cells of a male that only gave rise to non-yellow and fertile sons must have contained the extra marked Y chromosome; yellow and sterile sons indicate that they did not contain this extra Y chromosome, and when both phenotypes were found among the male progeny of a single male it could be assumed that such a phenotypically mosaic male was also germinally mosaic.

Out of a total of 260 phenotypically mosaic males obtained, six died shortly after their isolation and gave no offspring, 17 were sterile, six gave rise to less than 62 sons and 16 gave rise to some haplo-4 progeny. Of the remaining 215 mosaic males, 85 gave rise to *y* sons, 94 gave rise to *y*$^+$ sons and 36 to both *y* and *y*$^+$ sons. An average of 312 sons was observed per mosaic male and the smallest number of sons scored per male was 62. The data obtained from these 215 males made it possible to correlate the constitution of the germ cells to various cuticular landmarks and thus to determine their position on the genetic fate map of *D. melanogaster* (Fig. 1).

To rule out any sex-specific differences in the developmental origin of the germ cells and to obtain an independent corroboration of their posterior-most position (Fig. 1), an analogous investigation has been carried out with mosaic females. Males of the constitution *y*/*y*$^+$ *Y*; *pal*/*pal* were crossed to attached X *C(1)RM*, *y pn v*/0 females. The automatic virgin daughters obtained from this cross were screened for somatic mosaicism and their pattern of mosaicism was recorded. Subsequently, these mosaic females were crossed to *yellow* males. A total of 116 Y-chromosome mosaic females was obtained. The analysis, however, was based only on 105 mosaic females that gave rise to a minimum of 22 sons. The mean number of sons per female in this group was 98. Again, a fate map of cuticular landmarks was constructed and the location of the germ nuclei on this map was determined. Although there were some differences between this map and the map derived from mosaic males (Fig. 1), they were very similar in general outline and hence the map obtained from these 105 mosaic females is not reproduced here. Instead, the position of the germ cells in this map is superimposed on the map shown in Fig. 1.

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Cell lineage in retinal development of mice studied in experimental chimaeras

IN vertebrate embryos, the optic cups develop as lateral outgrowths of the diencephalon, the rudiments of which are first recognised at the antero-lateral margins of the neural plate. Evidence accumulating from extensive studies with vital dyes[1–4], excision and transplantation experiments[5–7] in amphibian embryos have shown that the cell boundaries of the different areas of the central nervous system are already fixed at the early neurula stage when the presumptive retinal cells are located in the antero-median border of the neural plate. It has remained unresolved whether the two retinal primordia and the layers thereof are determined in a single or separate group of cells. It seems to us that the problem is one of cell lineage and can be analysed with genetic mosaics. The retinal pigment epithelium (RPE) is derived from the outer layer of the embryonic optic cup. In chimaeric mice, produced by aggregating morulae from albino and pigmented strains, the presence of two types of cells in the RPE has been reported[8–10]. Deol and Whitten[11] first noted that RPE of the two eyes of such chimaeras showed similar proportions of cells. It was recently observed that there is a close correlation in occurrence of chimaerism in the RPE and in the neural retina and between the two eyes of the individuals[12,17]. Here we present quantitative data on the relative proportion and spatial distribution of cells from the two donor genotypes in the RPE and compare them with the relative proportion of cells in the neighbouring melanocyte population of the choroidal layer of the eye. The results show that there is a marked similarity in the proportion of the cells of the RPE from the two eyes of the individual chimaeras suggesting a lineage relationship between the two retinal primordia.

Chimaeric mice were produced by aggregation of morulae from albino BALB/c and pigmented C3H strains[12]. The eyes were removed when the animals were 20–26 days old and 8 μm thick serial sections were prepared. Out of 67 individuals obtained, 43 had both albino and pigmented cells in their RPE. Of these only 2 animals showed unilateral chimaerism, whereas in the rest the RPE of both eyes were chimaeric. Eyes from 20 animals, including the two showing unilateral chimaerism and representing a broad spectrum of distribution, were used in this study. The RPE from every third section was first drawn with a camera lucida and the stretches of albino and pigmented cells were marked. Each drawing was scanned in an x–y plotter attached to a computer. The program was designed to straighten the curved profiles of the RPE, to measure the linear lengths and calculate the relative proportions of the two components, and to map the spatial distribution of the cells in a two-dimensional reconstruction by aligning the linear outputs of the successive sections. In contrast to the RPE which is a single layer of cells, the choroidal melanocytes are located in a thick multicellular layer. For quantitative assessment every tenth section was used and the relative proportion of the albino cells in alternate stretches of the sections in the microscopic field were scored as 100% or 0% if completely albino or uniformly pigmented, 50% when the two kinds of cells appeared equal in proportion, 75% or 25% when the albino cells appeared to be relatively more or less than the pigmented cells; the mean value from all the scorings in each eye, numbering 150–180, was taken.

When the relative proportions of the albino cells in the RPE of the left and the right eyes from each chimaera are listed in the decreasing order (Table 1) it is seen that the two individuals with unilateral chimaerism represent the two extremes with 99.6% and 0.4% albino cells respectively. Among the 18 individuals with chimaeric distribution in both eyes, the relative proportions of the albino cells vary from 99.6% to 5%; but show a high degree of correlation between the two eyes in each of these chimaeras. In the melanocyte population of the choroidal layer unilateral chimaerism is seen in 6 individuals. In 4 other animals chimaeric distribution is seen in the RPE but the choroidal melanocytes of both eyes are non-chimaeric. In animals showing chimaeric distribution in both layers the relative proportion of the cells in the choroid often differs considerably from the relative proportion in the RPE of the same eye. The difference in the relative proportion of the cells in the choroid layer of the two eyes of the individual chimaeras is also considerably more than that of the RPE.

The spatial pattern of cell distribution in the RPE of the two eyes from 10 out of 20 chimaeras analysed is shown in Fig. 1. Although examination of serial sections reveals that stretches of albino and pigmented cells may vary from a single cell to one of many cells, in the reconstructed map of the entire RPE, it is clearly seen that the two kinds of cell are not randomly scattered though considerable intermingling has occurred. Areas of varying sizes with predominantly one type of cell could be seen in all cases and often such areas appeared as radial sectors stretching from the optic nervehead to the periphery. The number and dimension of such sectors varied among the individuals but in a few cases 10 alternate sectors with predominantly albino and pigmented cells, as reported previously[10], could be recognised (Fig. 1. 48 left eye, 45 right eye).

A similarity between the RPE of the two eyes could conceivably result from random assortment if the two types of cells were uniformly mixed throughout the chimaeric embryo. Observations on chimaeric blastocysts of mice, in which cells of one component were labelled with H^3-thymidine (ref. 13) and in early post-implantation embryos of mouse ⟷ rat chimaeras, in which the cells could be

Table 1 Relative proportions of albino cells (%) in the retinal pigment epithelium (RPE) and in the melanocyte population of the choroidal layer (CH) from the left and the right eyes of albino–pigmented chimaeric mice

Animal no.	Left eye RPE	Left eye CH	Right eye RPE	Right eye CH
23	99.6	100	100	100
2	99.6	100	99.3	88.1
16	98.8	100	98.2	100
8	98.5	84.7	98.1	100
10	97.2	88.2	90.8	100
49	96.4	100	95.0	100
56	95.6	98.7	95.7	93.8
29	95.5	100	95.2	97.8
63	93.2	94.9	89.6	100
48	85.6	99.1	77.3	88.9
69	82.7	82.2	72.0	94.3
9	78.8	87.5	72.5	91.6
45	75.9	74.0	77.0	67.8
18	66.9	77.2	61.6	60.8
3	58.8	70.1	72.0	83.9
1	49.8	76.1	38.8	58.1
54	32.5	30.1	37.0	63.3
20	13.3	24.5	12.1	56.0
44	5.0	14.9	7.5	0
14	0.4	0	0	0

The relationship between the percentage of albino cells in the RPE of the left and the right eye can be expressed by the following linear regression equation: $y = 0.8 + x$ (y, right eye; x, left eye) and the coefficient of determination (r^2) is 0.97. The difference between the RPE of the two eyes did not reach statistical significance (t dependent; $t = 1.4003$ d.f. 19). The absolute difference in the relative proportion of albino cells between the choroidal layer of the left and the right eyes is greater than that of the RPE (t dependent; $t = 3.226$ d.f. 19 $P < 0.005$ one-tailed).

identified by immunofluorescence[14], suggest that the cells from the two donor components are not intimately mixed but remain in coherent clusters. Transplantation of presumptive retinal cells in early stage *Axolotl* embryo has been shown to result in growth of the cells derived from the graft within well defined radial sectors of the RPE[15]. Although more random distribution of the cells in the RPE of 12½-day-old chimaeric mouse embryos in comparison to that of the adult RPE has been reported[16], there is no indication that at 12.5 d or at earlier stages of development cell mixture was uniform throughout the RPE. Localised distribution of pigmented cells in the RPE of animals with more than 95% albino cells, as observed in this study, makes it unlikely that cell mixture could have been uniform enough to ensure equal division by random process.

The bilaterality of cell distribution in the RPE of chimaeric mice, more particularly in the individuals showing preponderance of one of the cell types, suggests an orderly mechanism of cell deployment. It is concluded that the cells giving rise to the two optic cups are derived from

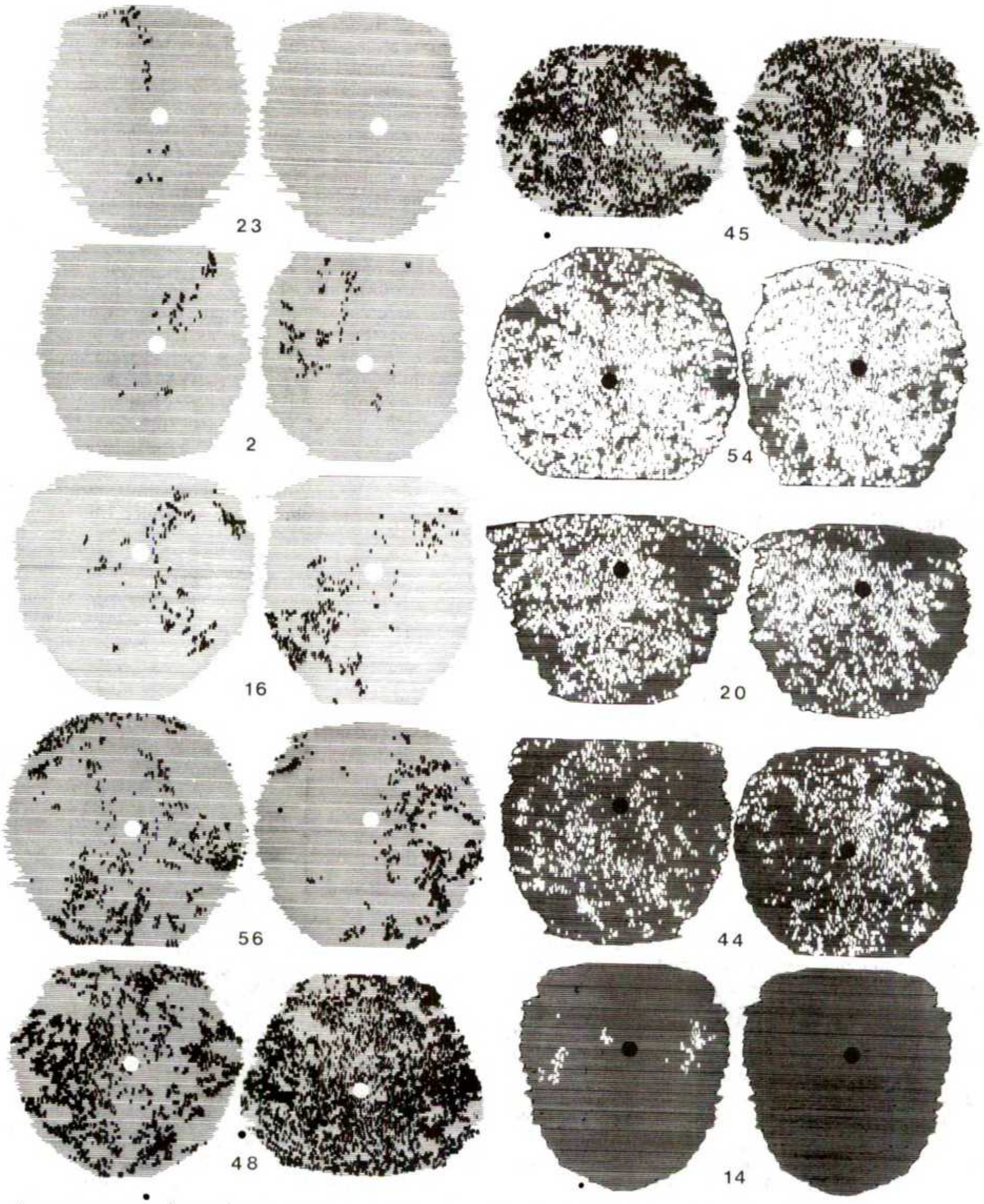

Fig. 1 Spatial distribution of albino (white) and pigmented (black) cells in the retinal pigment epithelium of the left and the right eyes from a series of chimaeric mice. The numbers indicate individuals listed in Table 1. The circle near the middle of each figure represent the optic nerve head.

common precursor cells, the lineage of which is so programmed that the daughter cells are segregated into two discrete lateral groups. It seems likely that such a mechanism may operate in the development of bilaterality of the central nervous system as a whole.

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Inductive capacity of irradiated dermal papillae

MAMMALIAN hair follicles are specialised skin appendages which produce a series of hairs, one after another, in a regular cyclical fashion. The hair itself is composed of dead epithelial cells containing large amounts of keratin. During growth of the hair the epithelial cells at the bottom of the follicle surround the dermal papilla and divide rapidly. The dermal papilla is derived from mesenchyme and is composed of non-dividing cells. The importance of the dermal papilla in maintenance and control of hair cycles has been assumed for many years but little direct evidence of its function has been demonstrated. Geary[1] caused permanent removal of rat body hair by applying 1,720 rad of X irradiation. He believed that he had caused destruction of the dermal papilla which in turn resulted in permanent epilation. We report here a study on the effect of heavy doses of irradiation on the dermal papilla and epithelial elements of the hair follicle. We have found that high doses of irradiation, which cause permanent epilation, destroy the epithelium but leave the dermal papilla anatomically and functionally intact so that even on transplantation it can induce a series of new hairs.

The mystacial vibrissal follicles of rats and mice form a remarkably constant pattern. Each follicle regularly produces a series of vibrissae with constant characteristics. Their lengths can be easily measured in the live animal by the capillary tube method[2,3]. The four largest vibrissal follicles, designated by Danforth[4] as E, F, G and H, form a vertical column in the most posterior position and the 'a' column of five slightly smaller vibrissal follicles are found immediately anterior.

A single dose of X ray was applied locally to the vibrissal follicles in young mice. Permanent epilation in the E, F, G and H column of follicles took place after a dose of 2,500 rad (ref. 5). Five months later these follicles were examined histologically and were found to have an isolated dermal papilla at the bottom of the follicle. This dermal papilla was rounded in shape and packed with cells. No epidermal cells were seen (Fig. 1). Permanent epilation was therefore considered to be a result of complete destruction of the epidermal constituents of the follicle while leaving the dermal papilla histologically intact.

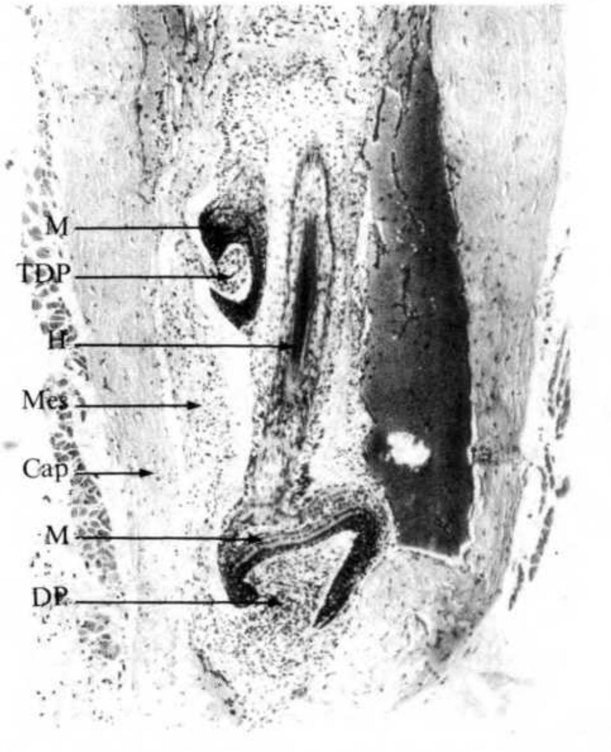
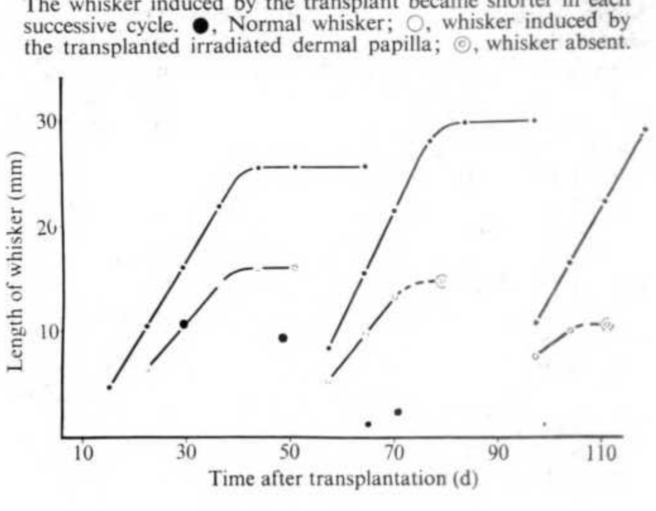


Fig. 1 A longitudinal section through a C_3H mouse (female) follicle given 2,500 rad of X irradiation. There is no trace of the matrix or other epithelial elements in the hair canal. (× 100.) DP, dermal papilla; BS, blood sinus; Cap, capsule.

To determine whether the dermal papilla in a heavily irradiated follicle is physiologically functional, it would be necessary to bring the irradiated dermal papilla into contact with normal follicular epithelium. To achieve this E, F, G and H vibrissal follicles in Wistar-derived rats were irradiated with 5,000 rad of X irradiation and on the next

Fig. 2 Growth curves of the two rat whiskers observed growing from Ia follicle after transplanting a heavily irradiated dermal papilla into its hair canal. The normal was a few mm shorter in the first cycle probably because of damage during transplantation. The whisker induced by the transplant became shorter in each successive cycle. ●, Normal whisker; ○, whisker induced by the transplanted irradiated dermal papilla; ◎, whisker absent.



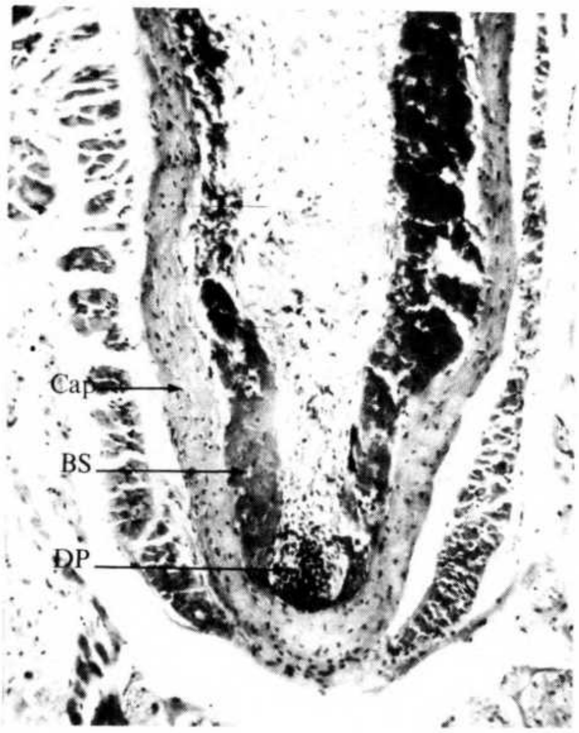


Fig. 3 The bottom end of an F follicle which received two dermal papillae irradiated with 5,000 rad. The vibrissae are inside the hair canal. The superior transplanted dermal papilla has made connection with the mesenchymal blood plexus. The second transplanted dermal papilla has distorted the normal at the bottom of the follicle and is not clearly shown in this section. (× 95.) Abbreviations as in Fig. 1; also M, matrix; Mes, mesenchymal sheath; H, hair induced by the host dermal papilla; TDP, highly irradiated, transplanted dermal papilla.

day the animals were killed and their dermal papillae were excised and transplanted into normal vibrissal follicles in normal rats. Three to four weeks later an extra whisker was observed growing alongside the normal vibrissa of the host follicle in approximately one third of the follicles. The whiskers induced by the irradiated dermal papillae were observed for two to three hair cycles before the rat either died or was killed. The induced vibrissae were shorter than the normal in length and had a slower rate of growth (Fig. 2).

Histologically, the induced hair bulb looked normal in most respects except for its situation (Fig. 3). We concluded that new hairs were induced by the transplanted dermal papillae wherever they were placed inside the hair follicle even if it was in the blood sinus. The length and size of the induced hair was dependent on the size of the transplanted dermal papilla. The whiskers produced by the irradiated and transplanted dermal papillae became shorter in successive cycles. They were more than 30% shorter at the end of three successive cycles.

Thus if heavily irradiated, permanently epilated follicles are left *in situ* the epithelium is destroyed but the dermal papilla remains as a recognisable but rounded mass of apparently viable cells. Transplantation of heavily irradiated dermal papillae into normal vibrissal follicles, however, induced additional new hairs which were observed for up to three successive cycles.

These findings show that a massive dose of X irradiation eliminates the epithelial elements of the growing hair, but does not abolish the inductive capacity of the dermal papilla. The failure of these dermal cells to display damage during at least 5 months observation following irradiation probably occurs because they do not divide; an analogous situation is found in the resistance to radiation of adult mammalian neurones[6].

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Vesicular disruption of myelin simulated by exposure of nerve to calcium ionophore

EXPERIMENTAL allergic encephalomyelitis (EAE) and experimental allergic neuritis (EAN) are autoimmune demyelinating conditions brought about by sensitisation to central and peripheral myelin extracts, respectively. The lesions are the result of a delayed hypersensitivity-type reaction which can be passively transferred by lymphoid cells but not by sera[1–4]. Demyelination occurs in areas of cellular infiltrate. This process is mediated in part by mononuclear cells which invade and phagocytise the target myelin tissue. A vesicular transformation of myelin sheaths is known to occur in close proximity to these invading mononuclear cells[5–7]. The nature of the myelin vesiculation is unclear; however, these changes have been emphasised as a characteristic accompaniment of autoimmune demyelination in experimental animals and in human peripheral nerve disease[8]. The present study reports the occurrence of the same type of myelin alteration in excised rat peripheral nerve fibres when incubated under conditions which promote a selective influx of calcium ions into the tissues.

Multiple 15-mm segments from the same excised and desheathed rat sciatic nerves were simultaneously incubated in oxygenated Ringer's solutions with and without the addition of A23187, 10 mg l^{-1}, and in calcium-free Ringer's solution containing ionophore and EGTA, 1 mmol l^{-1}, to chelate endogenous calcium. After 30-, 60- and 120-min incubations at 37 °C, the tissues were fixed by immersion and processed for electron microscopic examination as already described[9].

A vesicular disruption of myelin appeared as a distinctive alteration of nerve fibres incubated in the presence of both calcium and A23187. These changes were characterised by progressive vesicular transformation of myelin occurring along the surfaces of myelin sheaths, leading to increasing accumulations of a vesicular or foamy membranous meshwork adjacent to the remnants of original myelin sheaths. After 30 min, early vesicular changes could be seen as multiple vesicular eruptions along the inner and outer surfaces of compact myelin sheaths (Fig. 1). Progression of vesicular alterations was evidenced after 60 and 120 min by massive accumulations of a foamy material in myelinated fibres (Fig. 2). Vesicular alterations occurred predominantly in nerve fibres situated around the periphery of the nerve fascicles immediately beneath a thin residual perineurial investment (Fig. 3). The location of these fibres rendered them most susceptible to the effects of the incubation media as well as the fixative solutions. The penetration of lipophilic ionophore may also have been limited by the high lipid content of the nerve tissue.

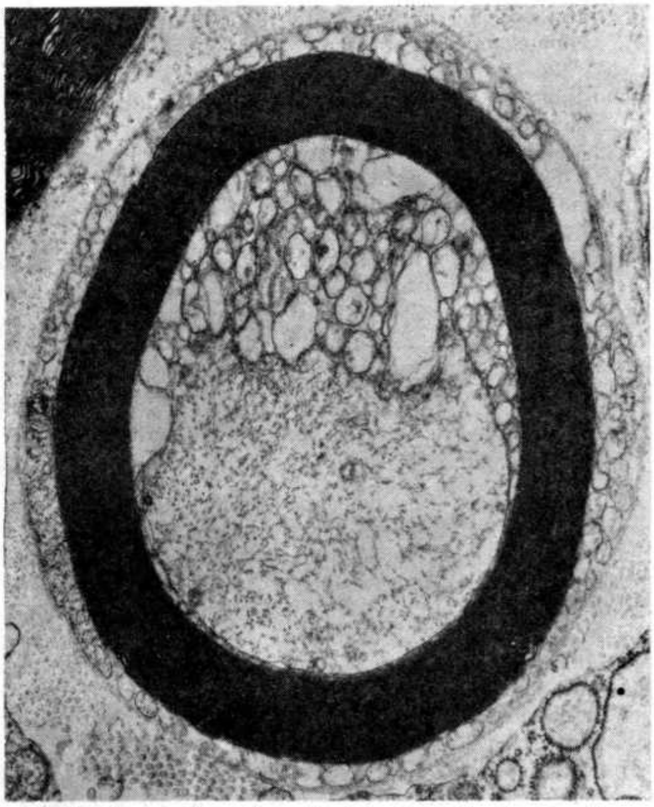
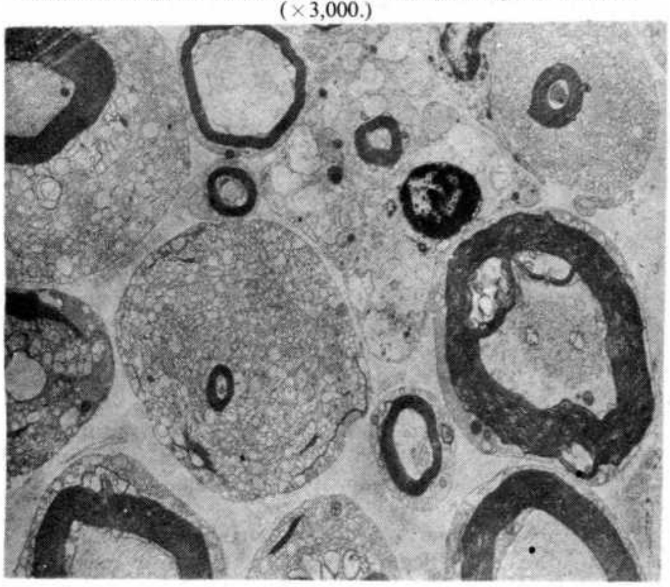


Fig. 1 Cross section of myelinated rat peripheral nerve fibre showing early vesicular disruption of myelin after 30-min exposure to A23187 (10 μg l^{-1}) in Ringer's solution containing $CaCl_2$ (4 mmol l^{-1}). Arrays of vesicular profiles occur along the inner and outer surfaces of the otherwise intact lamellar myelin sheath. (×14,000.)

A vesicular disruption of myelin was not seen in control nerve segments incubated in the absence of either ionophore or calcium. The vesicular type of myelin alteration was readily differentiated from preparative myelin artefacts which were scattered through the nerve fascicles and occurred with limited but equal frequency in experimental and control tissues. These myelin artefacts were characterised by concentric loosenings of myelin lamellae or by focal distortions or extrusions in the laminated cylindrical form of the myelin sheath.

Fig. 2 Cross section of nerve fascicle showing several fibres with more advanced states of myelin vesiculation following 120-min incubation in Ringer's solution containing A23187. A vesicular or foamy network has accumulated in Schwann cell cytoplasm adjacent to the residua of compact myelin sheaths. (×3,000.)



Incubations were conducted between *p*H 7.2 and 7.4, and visually monitored by inclusion of phenol red (1 : 10,000) in the media. Under these conditions, A23187 has selective high affinities for calcium and magnesium[10,11]. Both experimental and control incubations contained $MgCl_2$ (1 mmol l^{-1}), but only the experimental incubation contained both ionophore and calcium, a condition associated with the influx of calcium into tissues. The ability of A23187 to translocate calcium across cell membranes is well known and is believed to be due to a physical envelopment of calcium by ionophore, thereby rendering calcium lipid soluble and membrane permeable[12]. The direction of ionophore-mediated transmembranous movement of calcium is down the calcium concentration gradient. In calcium-free media containing EGTA, the calcium gradient is reversed. Incubation of nerve under these conditions acused separation of axolemmae from internal Schwann cell membrances of myelinated fibres, morphological features indicative of reduced tissue calcium observed following calcium deprivational conditions in tissue cultured peripheral nerve[13]. The absence of myelin vesiculation under these conditions suggests the existance of a subthreshold calcium level, insufficient to induce myelin vesiculation.

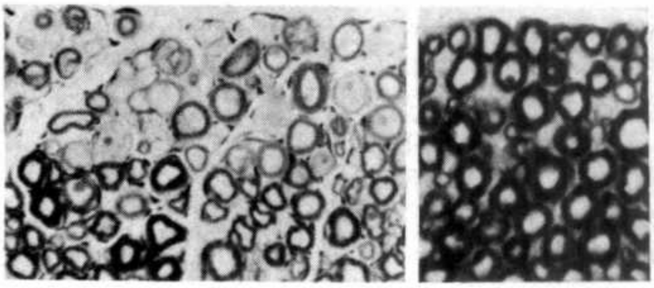


Fig. 3 Comparison of myelinated fibres along lateral edge of experimental (left) and control (right) nerve segments incubated for 120 min in Ringer's solutions with and without A23187. Superficial myelinated fibres in the experimental nerve segment reveal dissolution of compact myelin manifested by a loss in density of circular profiles, which represent cross sections of compact myelin sheaths. Additional control incubations in media containing ionophore but lacking calcium showed uniform preservation of compact myelin sheaths. Thin section of epoxy-embedded tissue stained with toluidine blue. (×450.)

These results imply the existence of calcium-reactive sites within the myelin sheath which induce myelin vesiculation upon their exposure to calcium. These calcium-reactive sites seem to be located within the myelin lamellae in such a manner as to render them inaccessible to calcium in the extracellular spaces in physiological conditions or to calcium present in the intraperiod space during incubations in hypotonic Ringer's solution[14]. Their relationship to calcium-binding sites of myelin[15] is unknown. Calcium-binding sites of plasma membranes may be exposed by detergent pretreatment[16]. The vesiculation of myelin which occurs in nerves exposed to lysophosphatidyl choline[17] could exemplify a detergent-induced exposure of calcium-reactive sites in the myelin sheath.

Myelin vesiculation which occurs in pathological conditions may also represent the manifestations of calcium interaction with components of the myelin sheath. Exposure of calcium-reactive sites could well occur during the degradation of myelin sheaths, resulting in occasional focal vesicular alterations reported or depicted in these circumstances[18–22]. The myelin vesiculation of cell-mediated immunological demyelination, however, is distinguished by its prominence and occurrence along the surfaces of intact myelin lamellae in the vicinity of infiltrating mononuclear cells[7-9]. The distribution of change in close proximity to these cells is strongly indicative of their complicity in the causation of myelin vesiculation. Numerous humoral factors have been identified as mediators of cellular immunological reactions[23] although the mechanisms underlying their activities have often remained obscure. A humoral mediator of myelin vesiculation would have particular interest in that its mechanism of action may well be operative by promoting calcium penetration into myelin membranes. A calcium influx is well recognised as a trigger for many biological phenomena. Accordingly, a humoral factor with the ability to increase calcium permeability of membranes could serve as a powerful means of effecting immunological reactions.

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Cancer-induced cytolysis of normal bone marrow cells

MOST investigations of the invasive process of cancer have involved microscopic studies of tumour spread after injection of cancer cells into experimental animals[1–4]. Attempts to investigate tumour invasiveness using organ or parabiotic cultures have met with limited success[5–7] We have evaluated tumour-mediated cytolysis of normal rat marrow cells using suspension cultures containing Walker-256 carcinosarcoma cells and ^{59}Fe-labelled rat bone marrow cells. This mixed cell culture system seems to provide a promising approach to the further understanding of cancer invasiveness.

Interactions between cancer cells and normal cells were evaluated by a modified cytotoxicity assay[8] Walker-256 carcinosarcoma cells (from Arthur D. Little Co. under the auspices of the NIH) were isolated from the ascites tumour which accumulated 7 d after intraperitoneal transplantation of 1 × 10^6 Walker-256 cells to adult Wistar rats. Tumour cells were separated from erythrocytes by centrifugation (400*g*) on a sterile gradient of a sucrose polymer and diatrizoate salts (LSM Solution, SG, 1.077–1.080, Litton). Tumour cells, located at the interface, were collected, washed twice and suspended in nutrient medium. More than 98% of the isolated cells were identified morphologically as tumour cells.

Rat marrow target cells were collected and prepared for suspension culture as before[9] Bone marrow cells (1 × 10^7 per ml) were labelled with 2 μCi ^{59}Fe per ml (ferrous citrate, Mallinckrodt) for 4 h at 37 °C. (After a 4-h incubation of rat marrow cells with ^{59}Fe-transferrin, Storring and Fatih reported that cytoplasmic ^{59}Fe accounted for approximately 78% and stromal ^{59}Fe for 22% of all ^{59}Fe incorporated[10]. Cytoplasmic ^{59}Fe consisted of haemoglobin iron (65%), ferritin iron 20% and an unidentified low molecular weight iron (15%)) The cells were washed four times to eliminate unincorporated label, suspended in nutrient medium (70% NCTC-135, 30% heat-inactivated foetal calf serum, Grand Island Biological, with penicillin and streptomycin) and added to various concentrations of Walker-256 cancer cells. The cell suspensions were mixed thoroughly by inversion and incubated in 10 × 75-mm friction cap tubes (Falcon) in 5% CO_2 and 95% air for 18 h at 37 °C. The mixed cell suspensions were then centrifuged at 400*g* for 10 min (4 °C), and the radioactivity in the supernatant and cell pellet was measured. Cytotoxicity is expressed as a function of the release index (RI)

$$\mathrm{RI} = \frac{\text{radioactivity released due to cell death (supernate)} \times 100}{\text{total radioactivity (supernatant and cell pellet)}}$$

Because most of the ^{59}Fe is taken up by haemoglobin-producing nucleated marrow cells (erythroblasts)[11], this technique measures principally the release of haemoglobin and non-haemoglobin iron-containing molecules from erythroblasts, and has been termed erythroblast cytotoxicity assay[12]. As in lymphocyte cytotoxicity studies[13], the baseline RI of marrow cells varied between experiments, making control cultures essential (^{59}Fe-labelled marrow cells alone) with each experiment. Significant differences between triplicate cultures of marrow cells alone and marrow cells cultured with Walker-256 cells were determined by Student's *t* test[14].

The ability of Walker-256 cancer cells to initiate marrow cell cytotoxicity is typified by the data in Table 1. Similar results were obtained in nine other experiments The spontaneous RI for marrow erythroblasts cultured alone was 18.5 ± 1.3% (s.e.m.) The increasing cytolysis of marrow cells cultured with Walker-256 cells was proportional to the ratio of tumour cells to marrow cells. In cultures containing equal numbers (1 × 10^7 cells) of Walker-256 and marrow cells the RI was 49.3 ± 1.2%. Marrow cell viability, as determined by Trypan blue exclusion, was inversely proportional to the number of tumour cells in culture. ^{51}Cr-labelling of rat marrow cells also resulted in significant, but less tumour-mediated marrow cytolysis

Table 1 Marrow cytotoxicity Trypan blue exclusion (viability) and RI

Ratio tumour cells to marrow target cells	RI (% ± s.e.m.)	Marrow viability (%)
0:1	18.5 ± 1.3	90
0.13:1	27.5 ± 1.0*	73
0.25:1	32.4 ± 1.0*	77
1:1	49.3 ± 1.2*	59
10:1	60.6 ± 1.4*	—
1 (alcohol-treated):1	17.9 ± 0.8	88
Tumour cell sap.:1 (from 1 × 10^8 cells)	18.3 ± 0.7	87
Ascitic fluid supernate:1	18.1 ± 0.8	76
1 Mouse spleen:1	21.0 ± 0.4	87
1 Rat spleen:1	18.9 ± 0.6	89

RI, release of ^{59}Fe from labelled rat bone marrow cells incubated for 18 h with viable Walker-256 carcinosarcoma cells, alcohol-treated Walker-256 cells, cell-free tumour ascitic fluid, or normal spleen cells

*$P < 0.01$, significantly different from control cultures containing ^{59}Fe-labelled rat marrow cells alone (0 tumour cells to 1 marrow cell)

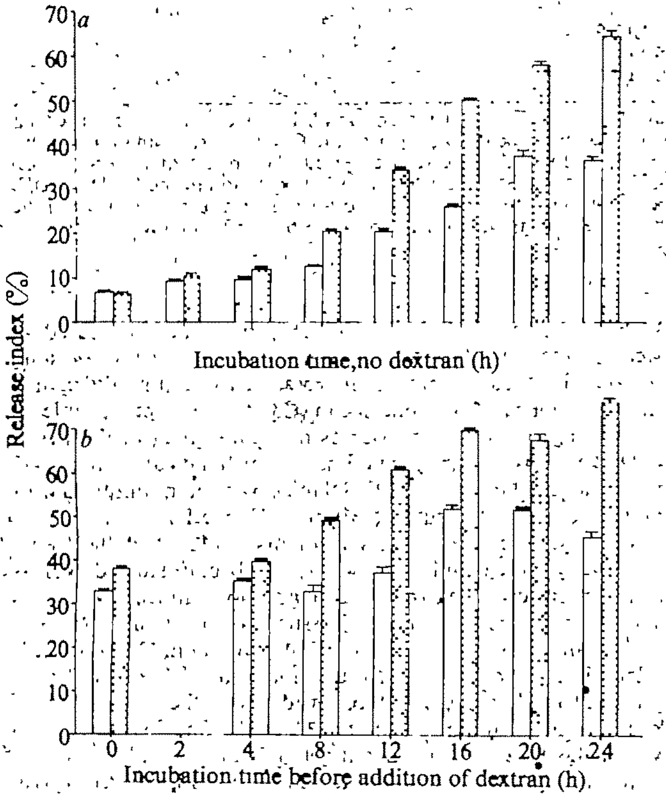
Tumour-mediated cytotoxicity of erythroblasts required intact tumour cells. No cytolysis was noted with alcohol-treated Walker-256 cells or the supernatant from the tumour ascitic fluid (Table 1) The cell sap of sonicated Walker-256 cells (100,000*g* for 90 min) also had no effect on erythroblast cytolysis When the effect of temperature on marrow RI was evaluated, no tumour-induced marrow cytolysis was observed in 24-h cell cultures maintained at 4 °C and was minimal at 25 °C. Maximum marrow cytolysis was observed at 37 °C.

To evaluate the ability of non-malignant cells to mediate marrow cytotoxicity, ^{59}Fe-labelled normal rat marrow cells were cultured with an equal number of normal mouse or rat spleen cells. Spleen cells did not significantly increase rat marrow erythroblast cytolysis (Table 1).

The progression of tumour-induced marrow cytolysis was evaluated by incubating tumour and marrow cells together and measuring RI at 0, 2, 4, 8, 12, 16, 20 and 24 h. As Fig. 1*a* shows, no significant increase in the release of ^{59}Fe from marrow cells was evident before 4 h, but marrow cytolysis increased during the next 12 h.

The requirement for intercellular contact between tumour cells and marrow cells during cytolysis was evaluated by a modification of Martz's method[8]. Equal numbers of Walker-256 cancer cells and ^{59}Fe-labelled marrow cells were incubated for 24 h in suspension cultures as described above. At specified intervals, however, 3 volumes of 10% high molecular weight Dextran (average 500,000. T-500, Pharmacia) in complete culture medium was added to each culture and mixed. Incubation was then completed and RI was measured. As Fig. 1 shows, erythroblast cytolysis increased as the duration of cell contact (before addition of Dextran) increased. When cell interaction was minimised by addition of high molecular weight Dextran at the initiation of culture (0 h), tumour-mediated marrow cytolysis was minimal.

Fig. 1. Cytolysis of ^{59}Fe-labelled marrow cells cultured alone (senescent lysis, clear bars) or with an equal number of tumour cells (senescent lysis and tumour-mediated lysis, shaded bars). *a*, Temporal progression of marrow cytolysis. Suspension cultures of marrow cells or marrow and tumour cells in nutrient medium were initiated at $t=0$ and terminated after 0, 2, 4, 8, 12, 16, 20 or 24 h of incubation. The cultures were centrifuged and the RI (mean ± s.e.m.) of triplicate cultures was calculated. *b*, Requirement for continuous marrow-tumour cell interation in order to obtain maximal tumour-mediated marrow cytolysis. Suspension cultures of marrow cells or marrow and tumour cells were initiated at $t=0$ and after 0, 4, 8, 12 16, 20, or 24 h of culture, 3 volumes of 10% Dextran in complete medium were added with vigorous mixing. Cultures were reincubated at 37 °C to give a total of 24 h of incubation. Cultures were then terminated and the RI of triplicate cultures was calculated. Minimal tumour-mediated marrow cytolysis was observed when Dextran was added within 4 h of the initiation of culture



Walker-256 cancer-mediated cytolysis of ^{59}Fe-labelled rat erythrocytes (labelled *in vivo* by intravenous injection of ^{59}Fe into rats 3 d before use of erythrocytes) was also demonstrated. The spontaneous release (RI) of ^{59}Fe from rat erythrocytes cultured alone was 1.5% in 20 h. The RI of rat erythrocytes cultured with Walker-256 cells (1:1 ratio) was 37.7%.

To determine whether the cytolytic interaction of Walker-256 tumour cells and erythrocytes was independent of phagocytosis, the phagocytic ability of Walker-256 tumour cells was evaluated by the method of Walker and Demus[15]. Tumour cells, collected and prepared as described above, were incubated for various times with erythrocytes labelled *in vitro* with ^{51}Cr. At the end of incubation the cells were centrifuged and the radioactivity in the supernatant (S_1) was determined. The cell pellet containing tumour cells, phagocytised ^{51}Cr-labelled erythrocytes and intact ^{51}Cr-labelled erythrocytes was suspended in distilled water and left at room temperature for 15 min. By this technique the engulfed erythrocytes would be protected from hypotonic lysis and ^{51}Cr would remain with the pellet. The cells were then centrifuged and the radioactivity in the supernant (S_2) and the pellet (P) was determined.

The phagocytosis index (%)

$$= \frac{\text{c.p.m. in pellet (P) after water lysis}}{\text{total c.p.m. } (S_1+S_2+P)} \times 100$$

was calculated. The phagocytosis of labelled erythrocytes by tumour cells was compared with the value obtained when ^{51}Cr-labelled erythrocytes, cultured without tumour, were subjected to hypotonic lysis, and the pellet-associated radioactivity was determined as described above. The phagocytosis index for erythrocytes cultured alone was 10.9±2.2% and for erythrocytes cultured with tumour was 12.6±0.5%. These values are not significantly different from each other ($P > 0.1$). Thus, in contrast to the murine reticulum cell sarcoma described by Ralph and Nakoing[16], Walker-256 breast carcinosarcoma is cytolytic both to nucleated and non-nucleated erythroid cells, but has no phagocytic capability in the system described above. Marrow–tumour cell cultures were also examined by electron microscopy, revealing no evidence of tumour phagocytosis of marrow cells.

Our findings indicate that Walker-256 carcinoma cells mediate the cytolysis of rat erythroblasts and erythrocytes in suspension cultures by a cell–cell interaction which is temperature-dependent and independent of phagocytosis. This cytolytic effect requires intact, viable Walker-256 cancer cells and, in contrast to lymphocyte-mediated cytotoxicity, will occur with a low effector cell-to-target cell ratio

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H–2^{a}- associated alloantigen expressed by several transplacentally-induced lung tumours of C3Hf mice

CELL surface changes accompanying malignancy may result in the expression by the tumour cells of antigens recognisable by the host's immune system. It is interesting to know whether nascent tumours express common tumour-associated antigens and what, if any, is the relationship of these antigens to histocompatibility alloantigens. We have addressed these questions by testing a series of early passage, transplacentally induced murine lung tumours for a cross-reactive histocompatibility locus-coded alloantigen. A common transplantation alloantigen was readily detected on six of 15 lung tumours tested.

According to the concept of immune surveillance, a major function of the immune system is the detection and elimination of nascent autochthonous tumours[1-3]. The cytotoxic T-cell-mediated immune response evoked in mice against allogeneic cells is directed primarily against cell surface components coded for by the K and D regions of the H–2 major histocompatibility complex (MHC) (ref. 4). The specificity of T-cell-mediated immunity against chemically modified[5,6] or virally infected[7-11] syngeneic cells is also determined by these MHC-coded components. Cytotoxicity mediated by T lymphocytes against certain long term transplanted murine leukaemias can be inhibited by antisera directed against the H–2 antigens expressed by the leukaemia cells[12,13]. We have demonstrated that the tumour-associated transplantation antigen on a transplacentally induced lung tumour of a C3Hf/HeN mouse is similar or identical to an antigen coded for by the K region of the MHC of strain A mice. Thus radioresistant syngeneic immunity can be induced against this tumour, designated 85, by immunising C3Hf mice with normal tissues of strains A, B10.A and B10.A (2R), but not C3Hf, B10 or B10.A (5R) mice[14]. These studies suggest that derepressed or altered components coded for by the MHC may provide the mechanism of recognition of nascent tumours by the cellular immune system. The MHC is subject to mutation[15] and antigenic deviation between the MHC-coded antigens expressed by a long term transplanted tumour and by the normal tissues of the mouse strain of tumour origin might occur during repeated passage of the tumour. Studies of the specificity of the MHC-coded antigens expressed by long term transplanted tumours cannot, therefore, be unequivocally extrapolated to the MHC-coded antigens expressed by nascent tumours. Therefore, we have studied 14 additional transplacentally induced lung tumours of C3Hf mice after either the 1st or 2nd *in vivo* passage for the expression of the strain-A-associated alloantigen detected on the 85 lung tumour

Lung tumours were induced by a single transplacental administration of 1-ethyl-1-nitrosourea (ENU) to pregnant C3Hf mice (0.5 µmol per g body weight). The mice were injected between days 13 and 16 of gestation. Progeny mice were killed at approximately 1 yr of age and lung tumours greater than 5 mm in diameter were transplanted as fragments into syngeneic C3Hf mice. Fourteen tumours grew on 1st passage. Some of the tumours were retransplanted into a second group of syngeneic recipients. Tumour tissue from either the 1st or 2nd *in vivo* passage was collected and cut into approximately 1-mm square fragments. These fragments were stored in a 10% dimethyl sulphoxide solution under liquid nitrogen. Groups of C3Hf and (C3Hf × A)F$_1$ hybrid mice were inoculated intradermally with a single fragment of tumour as described previously[15,16]. Tumours which grew were excised from the tumour-inoculated mice. Control C3Hf mice were inoculated with a lung tissue fragment from either strain B10.A (H–2^{a}) or B-10 (H–2^{b}) mice. Immunised mice were X-irradiated (400 R) and challenged with 10^{6} 85 tumour cells. Tumour growth was measured at weekly intervals. Preimmunisation with five of the 14 lung tumours induced radioresistant cross-reactive immunity against the 85 tumour in C3Hf but not in (C3Hf × A)F$_1$ mice (Table 1). The linkage of the 85 tumour associated antigen to the MHC of H–2^{a} mice is shown by experiments which indicate that anti-85 immunity can be induced in C3Hf mice by preimmunisation with lung tissue of B10.A but not B10 mice (Table 1).

The results indicate that six out of 15 (40%) of the transplacentally induced lung tumours of C3Hf mice studied express a common cross-reacting transplantation antigen which is similar or identical to a normal tissue alloantigen of H–2^{a} mice. The finding of this common transplantation, MHC-coded, alloantigen is consistent with the important role attributed to modified or derepressed MHC products in the recognition of nascent autochthonous tumours[3,12,13,16,17]. The frequency with which the H–2^{a}-associated antigen is expressed also suggests that a clearly definable alteration in genetic regulation or syn-

Table 1 Ability of six transplacentally-induced lung tumours of C3Hf mice to induce radioresistant immunity in C3Hf, but not in (C3Hf × A)F$_1$ mice, against the 85 tumour

Pre-immunisation	Tumour growth in X-irradiated mice			
	C3Hf mice		(C3Hf × A)F$_1$ mice	
	Tumour incidence	Mean tumour diameter ± s.e. (mm)	Tumour incidence	Mean tumour diameter ± s.e. (mm)
Nil	10/10	10.8 ± 1.1	8/8	15.1 ± 1.0
85	1/9	0.3 ± 0.3*	8/8	12.0 ± 1.6
38	1/10	0.4 ± 0.3*	8/8	12.3 ± 1.4
292 F-3	3/10	3.9 ± 1.8*	9/9	17.3 ± 1.0
294 F-2	1/10	0.4 ± 0.4*	9/9	16.4 ± 0.8
320 M-1	2/9	3.3 ± 1.3*	10/10	15.0 ± 0.8
321 M-3	7/10	4.9 ± 1.1*	9/9	14.0 ± 0.6
Other tumours (9 total)	88/89	10.3 10.8 11.3 12.3 12.6 12.7 13.2 14.1 15.7	NT	NT
B10 lung	8/8	12.5 ± 0.8	NT	NT
B10.A lung	0/8	0.0 ± 0.0	NT	NT

Palpable tumours were present at 1 week after tumour inoculation in all (C3Hf × A)F$_1$ recipients and in all but one C3Hf recipient which received tissue from the nine non-cross-reacting lung tumours As indicated by tumour incidence, most of the C3Hf mice pre-immunised with tissue from the six cross-reacting lung tumours failed to develop tumours during a 2-month observation period. The mean diameters (±s.e) recorded here were those obtained 4 weeks after tumour inoculation in the C3Hf mice and 3 weeks after tumour inoculation in the (C3Hf × A)F$_1$ mice. Results marked with an asterisk indicate a significant difference from tumour growth in that group compared with tumour growth in the group not pre-immunised ($P < 0.001$). The other values were not significantly different from the control group ($P > 0.5$). NT, not tested.

thesis of the MHC-coded components is associated with lung tumour development in C3Hf mice.

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Antibody-enhanced dengue virus infection in primate leukocytes

DENGUE viruses, types 1–4, are arthropod-borne flaviviruses which cause dengue shock syndrome (DSS) in humans possessing pre-infection antibody, passively acquired or derived from heterotypic infection[1]. Although the immunopathological mechanism of DSS is not fully understood, experimental studies suggest that dengue virus-production is immunologically regulated. Monkeys with monotypic immunity to dengue types 1, 3 or 4 viruses, when challenged with dengue 2, had significantly higher levels of circulating virus than did similarly infected susceptible animals[2]. This may be attributable to the replication of virus in leukocytes. In infected monkeys, virus was frequently recovered from buffy coat cells and from lymphatic tissues[3]. The role of leukocytes in enhanced infection is further supported by the observation that dengue replicates readily in cultures of peripheral blood leukocytes (PBL) prepared from immune simian or human donors, but poorly or not at all in leukocytes from non-immune hosts[4–6]. Studies on the immunological specificity of this phenomenon have been hindered by the requirement either for expensive experimental hosts (monkeys) or for human donors with chronologically defined dengue infections. Here we describe the *in vitro* enhancement of dengue infection in PBL by antibody. This system provides a provisional model for DSS in young infants during primary dengue infections[7,8].

Dengue virus, type 2 (strain 16681) (D2V) propagated in a continuous rhesus kidney cell line, LLC-MK2, was used for all *in vitro* infections[9]. D2V seeds contained approximately 10^7 plaque-forming units (PFU) per ml.

Dengue antibodies were obtained from man, monkey, mouse and rabbit. Serum was collected from naturally infected humans, or raised in monkeys by bleeding animals one or more months after a single subcutaneous injection of one of four prototype tissue culture passaged dengue viruses[9]. This regularly induced infection with the production of antibodies whose serological characteristics have been described[10]. In mice, antibodies were raised by a standard immunising schedule, using prototype mouse adapted dengue strains[11]. Antibodies were obtained in ascitic fluids induced by sarcoma 180 cells[12]. In rabbits, dengue antibodies were raised as described by DeMadrid and Porterfield[13].

PBL were collected from humans, *Macaca mulatta*, *Macaca irus*, New Zealand rabbits, Duncan Hartley guinea pigs and several mouse strains. Sera from each leukocyte donor failed to neutralise dengue 1, 2, 3 and 4 viruses when measured at a 1:10 dilution in the LLC-MK2 assay[9]. PBL were separated from erythrocytes using dextran T-250 (refs 4–6) or by isopyknic centrifugation on Ficoll-Hypaque[14]. Viable cells were adjusted to either 1 or 1.5×10^6 mononuclear leukocytes per ml in RPMI-1640 supplemented with 10% heat-inactivated (56 °C for 30 min) foetal calf serum (FCS), 20 mM HEPES, 0.02% $NaHCO_3$, 2 mM glutamine and 200 U ml^{-1} pencillin and 200 $\mu g\ ml^{-1}$ streptomycin. In some experiments heat-inactivated autologous or pooled normal monkey serum was substituted for FCS.

Dengue virus at a multiplicity of infection (MOI) of 0.1–0.01, was added to PBL suspensions containing various concentrations of dengue antiserum and to control cultures without dengue antiserum. Below a MOI of 0.001, infection did not usually occur. At first, virus, serum and PBL were incubated for 1 h at 37 °C, cell suspensions were washed twice in 50 volumes of Hanks' BSS then resuspended in medium at 1×10^6 cells per ml. Cells were incubated in screw-capped glass or plastic vials at 37 °C. Later, when

Fig. 1 Dengue 2 virus titres in cultures of normal rhesus peripheral blood leukocytes (PBL) in 20 paired comparisons, with (●) and without (○) anti-dengue 4. D2V was added to all cultures at MOI of 0.2–0.05. Virus was not removed by washing. To one half of cultures a single lot of anti-dengue 4 was added at a final dilution of 1:30. Control cultures were incubated with 1:30 autologous serum or pooled normal monkey serum. Mean plaque forming units were calculated from three 1-ounce LLC-MK2 bottles; averages of means are plotted ± s.e.m. The *t* test for paired data was performed using the square roots of mean plaque counts.

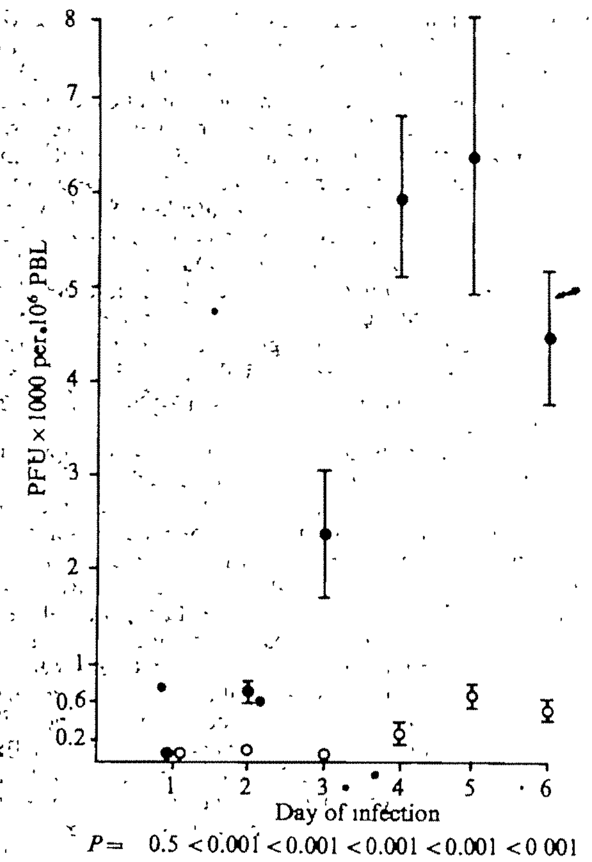

Table 1 Dengue 2 virus infection enhancement activity of serial 10-fold dilutions of anti-dengue sera raised in man, monkey, mouse and rabbit

Antibody specificity	Antibody donor species	PBL donor species	Reciprocal of titre: Against D2V HI	Reciprocal of titre: Against D2V PRNT	Reciprocal of titre: Against homologous virus* PRNT	PFU $\frac{\text{serum}}{\text{control}}$ at indicated dilution† 10^{-1}	10^{-2}	10^{-3}	10^{-4}
anti-D1	*M. mulatta*	rhesus	20	20	640	*63*	*123*	*14*	1
-D2	*M. mulatta*	rhesus	320	1,280	—	<1	<1	*9*	*101*
-D3	*M. mulatta*	rhesus	40	15	320	*169*	*26*	1.6	<1
-D3	rabbit‡	man	ND	320	160	*6*	*13*	*10*	1.4
-D4	*M. mulatta*	rhesus	40	12	640	*16*	*28*	1	1
-D4	man§	rhesus	10	<10	180	*32*	*9*	<1	<1
-D4	mouse	rhesus	40	18	320	*12*	*29*	*12*	1

*Serial four-fold dilutions of serum were tested against 5–40 (PFU) of homologous virus in a LLC-MK$_2$ plaque assay. Tests involved haemagglutination–inhibition (HI) or a plaque reduction neutralisation test (PRNT).

†Ratio between PFU on day 4 of rhesus PBL cultures inoculated with D2V (MOI = 0.1) plus anti-dengue (serum) divided by PFU in control cultures. Significant differences ($P < 0.05$) are shown in italics.

‡Serum described by De Madrid and Porterfield[13]. Tested in human PBL and virus in day 3 cultures compared.

§Serum of G. Caron Sr. (ref. 15).

it was established that input dengue virus decayed to below detectable levels at 48 h, the washing step was omitted[8]. Virus was assayed on LLC-MK2 monolayers by a plaque method[9]. Infection enhancement was defined as a significant increase in plaque counts observed in antibody-containing cultures compared with appropriate control groups (*t* test).

Comparative D2V growth observed in 20 paired experiments using rhesus PBL is shown in Fig. 1. In *M. mulatta* (rhesus monkey) or *M. irus* PBL cultures, virus titres were maximal after 72–120 h of culture but in human PBL peak virus growth occurred between 48 and 72 h. The enhancement potencies of selected dengue 1, 2, 3 or 4 antisera prepared in various vertebrate species are illustrated in Table 1. For each serum, neutralising antibody titres against homologous and dengue 2 viruses measured in LLC-MK2 cells are compared with D2V infection enhancing titres in PBL. Anti-dengue 1, 3 and 4 sera raised in man, monkeys, rabbits or mice produced a similar degree of D2V infection enhancement in monkey or human PBL over a broad dilution range. Monkey anti-dengue 2, with a homologous 50% plaque reduction neutralisation titre of 1 : 1280, neutralised D2V in PBL cultures at 1 : 100 but when diluted to and above the neutralisation end point, enhanced infection. Infection enhancement titres with homologous antisera were usually 20–100-fold greater than neutralisation titres; those in heterologous sera were usually 50–500-fold higher than the D2V neutralisation titre.

Over a period of 3 yr, PBL from 40 non-immune humans of all ages, from 25 *M. mulatta* and 22 *M. irus* have demonstrated D2V enhancement, but PBL obtained from two adult New Zealand rabbits, two guinea pigs and from two adult mice of each of eight strains (Swiss Webster, AKR, TO, DBA/TO, $C_{57}Bl_{10}$, Balb C, C_3H and DBA_2) failed to support replication in the presence of dengue 1–4 antibodies.

We have obtained evidence that the leukocyte supporting dengue replication *in vitro* is a mononuclear phagocyte (unpublished results). Thus, enhancement of dengue virus infection may be a special case of the opsonisation phenomenon Non-neutralising antibody presumably provides a specific molecular 'ride' for an infectious dengue virion into the interior of a receptive phagocytic cell. It is well established that mononuclear phagocytes may serve as a genetically controlled determinant of viral pathogenicity, may be the initial site of viral replication, and may be a vehicle for systemic spread of and definitive host for viruses[16]. The failure of mouse, guinea pig and rabbit PBL to support dengue virus replication correlates with the insusceptibility of these species to dengue virus infection by the peripheral route[17] and may provide yet another example of pathogenicity determined by the ability of a virus to replicate in phagocytic cells. Mononuclear phagocytes are thought to be the only cells supporting replication in several viral infections, including Aleutian disease of mink[18]. Of particular interest to our report is the speculation that disease infection of monocytes results from the internalisation of virus-aitibody complexes[18].

If it is assumed that the right type and amount of dengue antibodies can increase the number of dengue-infected leukocytes *in vivo* and if enhanced cellular infection relates directly to disease severity, then the unusual age distribution of DSS in infants can be easily explained by the *in vitro* model described here. DSS is rarely seen in the first 3 months of life, then incidence rates rapidly increase, peak at 8 months and wane to low levels at 12 months (ref. 7). This suggests that in man as in PBL cultures, high concentrations of maternal dengue antibody may neutralise invading virus and protect the infant, but that after several months as antibody levels fall, infection enhancing activity predominates. In time this activity is lost.

The observation of antibody-enhanced virus infection in primate leukocytes is apparently new. Antibody enhancement of virus plaques, however, is well established in several systems. Hawkes and Lafferty noted enhanced plaque production when Murray Valley encephalitis virus (MVE) and rabbitpox viruses were inoculated on chick embryo fibroblast (CEF) monolayers in the presence of high dilutions of homotypic antisera[19]. Infection was mediated by avian IgG antibody and was completed within 30 min after virus-antibody mixtures were added to cell sheets. Mammalian antisera, although similar in potency, did not enhance. This unexplained result resembles a phenomenon described by Kjellén and Schlesinger[20]. In their study, mixtures of vesicular stomatitis virus (VSV) plus diluted anti-VSV inoculated on CEF, resulted in titres 10–1,000-fold higher than when the same mixtures were applied to a continuous cell line. We found that CEF contain large numbers of phagocytic cells. Hence, enhancement of plaques on CEF and perhaps some instances of 'persistence' of virus infectivity in high dilutions of antisera may be earlier descriptions of the phenomenon reported here. In a related experiment, Azuma observed enhanced interferon production in mouse macrophages incubated with mixtures of Newcastle disease virus (NDV) and anti-NDV at dilutions above the neutralisation endpoint[21]. Virus replication was not measured, but interferon production was mediated by IgG, independent of complement, and required an active Fc terminal.

These reports suggest that enhanced viral infection of phagocytic cells by non-neutralising antibody may be a rather general biological phenomenon. This possibility should be explored, particularly with viruses which exist in antigenically related groups.

We are now identifying immunoglobulin(s) mediating infection in PBL, studying the conditions which affect the reproducibility of *in vitro* antibody-mediated infection enhancement and the mechanism of infection of leukocytes by antibody-virus complexes. The availability of a simple system in which to elicit antibody-enhanced leukocyte infection may clarify the role of dengue-infected leukocytes in human pathogenic reactions.

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Colchicine inhibits phosphatidylinositol turnover induced in lymphocytes by concavalin A

RECENTLY colchicine has been shown to affect various membrane properties such as the lateral mobility of receptors and other surface membrane markers and the agglutination of different cell types. Many of the described phenomena also involve concanavalin A (con A); for example, phagocytosis by rabbit polymorphonuclear leukocytes (PMNs) leads to a selective depletion of con A binding sites which is prevented by colchicine[1], con A inhibition of lymphocyte immunoglobulin receptor capping is reversed by colchicine[2–4] and con A-induced aggregation of PMNs, fibroblasts and hepatoma cells is inhibited by colchicine[5–7]. In addition to these effects on membrane properties, colchicine also has been shown to inhibit con A-induced lymphocyte transformation at an early stage in the sequence of events leading to thymidine incorporation and blast transformation[8,9]. These various considerations led us to determine if colchicine could alter phosphatidylinositol (PI) turnover in lymphocytes since it is known that increased PI turnover is an early event induced by con A and other mitogenic lectins[10–12].

Table 1 shows that colchicine inhibits both unstimulated and con A-stimulated ^{3}H-inositol incorporation into PI in a dose-dependent manner. At a concentration of 10^{-6} M the drug inhibited the con A increase in incorporation by 40% to 66%. In other experiments (data not shown) 3×10^{-7} M colchicine caused slight but insignificant inhibition while at concentrations of 10^{-7} M or below it had no discernible effect. Colchicine also inhibited inositol incorporation into PI in unstimulated cells although to a lesser degree than when con A was also present. At a concentration of 10^{-6} M colchicine inhibited this basal incorporation in two out of four experiments (experiments 1 and 2, $P<0.01$ and 0.1 respectively, Student's *t* test). At higher concentrations inhibition was both more pronounced and more consistent. Lumicolchicine was studied in one experiment only. At the concentrations used (10^{-6} M and 10^{-5} M) it had no effect on either basal or con A-stimulated inositol incorporation (data not shown).

While the results in Table 1 are very clear neither the mechanism by which colchicine inhibits inositol incorporation into PI nor the possible significance of this observation are immediately obvious. With respect to mechanism, it is well established that colchicine binds to tubulin and thereby prevents the formation of microtubules. It is possible, therefore, that an intact microtubule system is required for phosphatidylinositol turnover to occur. But it has been known for several years that colchicine also binds to particulate fractions in several cell types[13–16] and that in the brain and thyroid gland, at least, the characteristics of this binding are indistinguishable from those of colchicine binding to tubulin. In addition, freeze-etch electron microscopy has shown that colchicine impairs the mobility of constituents of the alveolar membranes from *Tetrahymena pyriformis*[17], presumably without altering microtubules. We favour the hypothesis that colchicine inhibits inositol incorporation through a direct interaction with tubulin or tubulin-like protein in membranes. It should be noted that this distinction between a colchicine action on microtubules or on membrane-bound protein may soon become irrevelent since there is increasing evidence of a very close association between microtubules and membranes. In rabbit ovarian granulosa cells, for example, microtubules are seen perpendicular to and directly beneath con A caps while fluorescein-labelled colchicine is localised to the cap region[18]. Similarly, Berlin and his colleagues have recently applied the technique of resonance energy transfer between fluorescent chromophores to demonstrate a close association between tubulin and membrane components in rabbit polymorphonuclear leukocytes[19,20].

Another possible explanation for the present results, namely that colchicine directly affects an enzyme or enzymes involved in PI turnover, seems unlikely since colchicine has been shown not to affect the enzymatic activity of a soluble PI phosphodiesterase purified from hog thyroid gland[21]. It is of considerable interest, nonetheless, that this phospholipase C-type enzyme, which forms cyclic inositol phosphate as one of its products, has been shown to be associated with tubulin[21,22]. Finally, it should be mentioned that the trivial explanation, inhibition by colchicine of con A binding, has been eliminated by other investigators[8].

Before considering the relevance of the present observations it is necessary to consider the significance of increased inositol incorporation into PI. It has been known for many years that numerous stimuli cause the increased incorporation of both phosphate and inositol into phosphatidylinositol (for review, see refs 23–25). In almost all tissues this incorporation reflects the turnover of the phosphorylinositol portion of the PI molecule rather than *de novo* synthesis. The enzyme initially activated by the stimuli which lead to PI turnover is of the phospholipase C type and is capable of forming D-myoinositol 1,2-cyclic phosphate as one of its water-soluble products[26,27]. Four years ago Michell and Lapetina suggested that this compound acts as an intracellular second messenger[27]. Increased PI turnover occurs almost immediately after the addition of con A or other

Table 1 Effects of concanavalin A and colchicine on ^{3}H-inositol incorporation into PI

Additions		Incorporation (counts per 10 min ± s.d.) Expt 1	Expt 2	Expt 3	Expt 4
—		1,005±44	679±34	1,212±56	1,962±198
Colchicine	10^{-4} M	823±130	358±41	751±54	1,191±42
Colchicine	10^{-6} M	910±49	485±56	1,159±58	1,450±193
Colchicine	10^{-8} M	854±21	540±83	1,258±73	2,039±153
Con A	20 µg ml^{-1}	1,707±77	1,129±12	2,860±196	3,581±166
Con A+colchicine	10^{-4} M	1,089±59	555±56	1,131±37	1,860±48
Con A+colchicine	10^{-6} M	1,200±180	767±71	1,599±17	2,250±26
Con A+colchicine	10^{-8} M	1,273±160	829±74	1,893±113	2,740±212

Four to five × 10^6 human peripheral lymphocytes isolated by density gradient centrifugation were incubated in triplicate in 1.0 ml Eagle's minimum essential medium containing 10% foetal calf serum with or without colchicine for 30 min at 37 °C. Con A and ^{3}H-inositol (approximately 1 µCi) were added and the incubation continued for 100 min after which the cells were pelleted and washed twice with medium. After addition of 7.5 ml chloroform–methanol (1:2) the cells were sonicated. 2.5 ml each of H_2O and chloroform were then added and the samples left overnight. Following centrifugation the chloroform phase was dried in scintillation vials, the residue dissolved in scintillation solution and the ^{3}H content determined in a liquid scintillation counter. The ^{3}H was shown to be localised to the PI fraction by ascending chromatography using silica gel loaded paper and both basic and acidic solvents. In preliminary experiments con A-enhanced ^{3}H-inositol incorporation into PI at concentrations of 2.5–20 µg ml^{-1}. 20 µg ml^{-1} con A was used in subsequent experiments since it produced the greatest enhancement without causing any cytotoxicity as determined by Erythrocin B exclusion.

mitogenic, but not non-mitogenic, lectins to lymphocytes and continues for as long as the lectin is present[10,11]. It has been proposed that this enhanced PI turnover is an essential step in the mechanism of lymphocyte activation. We consider that the present demonstration of colchicine inhibition of PI turnover both explains the recent observation[8,9] that colchicine inhibits concanavalin A-induced lymphocyte transformation at an early stage and also provides additional strong support for the hypothesis that increased PI turnover is required for lymphocyte transformation to occur.

The significance of the present observation with respect to colchicine effects on lateral mobility within membranes and cell agglutination is difficult to assess at present. Both phagocytosis, which initiates movement within membranes[1,28], and anti-immunoglobulin antisera are stimuli which increase PI turnover in PMNs[29–31] and lymphocytes[12] respectively. It is certainly possible that membrane changes influencing mobility occur simultaneously with or as a later result of this increased PI turnover and that colchicine alters movement by its effect on this parameter.

In summary, colchicine has been found to inhibit both basal and concanavalin A-stimulated incorporation of ^{3}H-inositol into phosphatidylinositol. We consider this effect explains the early inhibition by colchicine of con A-induced thymidine incorporation into lymphocytes and has possible relevance for colchicine effects on membrane mobility and agglutination. It will be important to determine if this newly described action of colchicine occurs in cell types other than lymphocytes and if it is shared by other microtubule-active agents.

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Asymmetrical distribution of microvilli in cytochalasin B-induced pseudocleavage of mouse oocytes

CYTOCHALASIN B, a fungal alkaloid first described by Carter[1], has diverse effects on cultured cells, including inhibition of morphogenetic and movement processes, and interference with hormone mediated cellular response[2]. It also induces various cultured mammalian cells to segregate the nucleus into an evagination of the plasma membrane which occasionally breaks away from the cell body[3]. Although some of the biological effects of cytochalasin B have been attributed to the disruption of microfilamentous structures found in cells, the mechanism of action of the drug remains unclear[4]. We have found that mouse oocytes cultured in the presence of cytochalasin B divide into two compartments; a process we called 'pseudocleavage'[5]. We now report, using scanning electron microscopy, that although one of the oocyte compartments is covered with microvilli, the other is smooth. This suggests that cytochalasin B triggers a redistribution of cell surface elements in the mouse oocyte.

Approximately 15% of mouse oocytes cultured for 16 h in medium containing cytochalasin B (CCB, 5 µg ml^{-1}) underwent pseudocleavage (Fig. 1*a* and *d*). This percentage was increased several-fold by the addition of db cyclic AMP (0.2 mM) to the medium[6] or by using oocytes which failed to resume meiosis when cultured for 3 h in medium without CCB (Fig. 2). The latter experiments show that exogenous db cyclic AMP is not required for CCB-induced pseudocleavage.

To compare the topography of normal and pseudocleaved oocytes, isolated mouse oocytes were cultured in the presence of either db cyclic AMP (0.2 mM) alone or db cyclic AMP (0.2 mM) and CCB (5 μg ml^{-1}) for 16 h, then fixed and processed for scanning electron microscopy. The surface of oocytes cultured in the presence of db cyclic AMP alone was uniformly covered with microvilli (Fig. 1*b* and *c*), resembling that of oocytes cultured without db cyclic AMP. On the other hand, the surface of one of the two compartments resulting from pseudocleavage was covered with microvilli, while the other was comparatively smooth (Fig. 1*e* and *f*).

Because microvilli are uniformly distributed on the surface of mouse oocytes cultured in the presence or absence of db cyclic AMP, pseudocleavage apparently involves extensive topographical reorganisation of the oocyte. Although the mechanism of action of CCB is not clear[1], we suggest that the surface asymmetry observed here is due to interaction of CCB with the oocyte's cortical contractile apparatus[10]. In this connection, several investigators have suggested that peripheral membrane

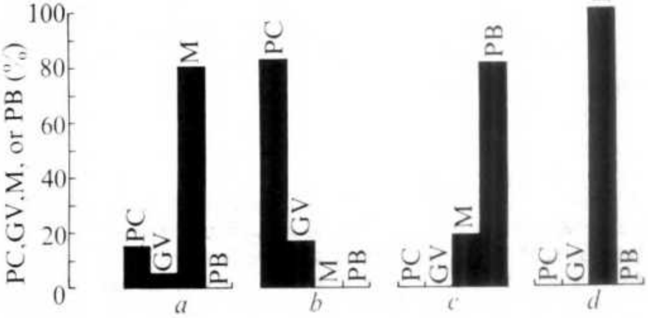

Fig. 2 Analysis of oocyte behaviour in different culture conditions. In all cases oocytes were cultured for 16 h then scored microscopically as pseudocleaved (PC), germinal vesicle (GV), metaphase I (M), or polar body (PB) oocytes. *a*, oocytes cultured continuously in the presence of CCB (5 μg ml^{-1}). *b–d*, oocytes were cultured in medium for 3 h, then those retaining an intact GV were transferred to medium containing CCB (5 μg ml^{-1}) (*b*), and oocytes which had undergone GV breakdown were transferred either to medium (*c*) or to medium containing CCB (5 μg ml^{-1}) (*d*). Each sample contained 50 or more oocytes.

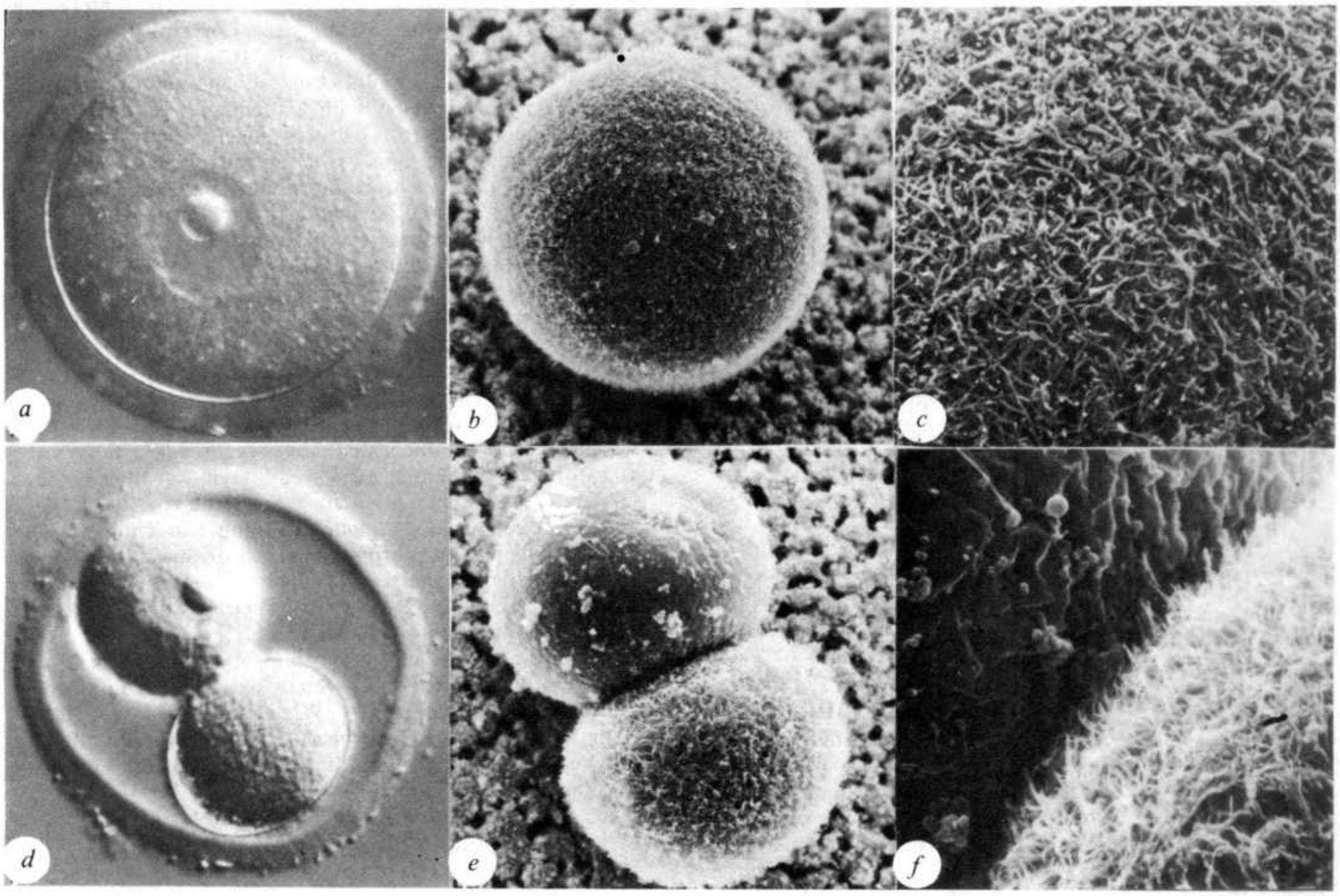

Fig. 1 Photomicrographs of mouse oocytes after *in vitro* culture. Oocytes were obtained by puncturing ovaries dissected from adult (8–12 weeks old) virgin female Swiss mice (CD-1, Charles River)[6]. Oocytes containing an intact germinal vesicle (GV) and free of cumulus cells were collected and washed in a chemically defined medium[7] and cultured in this medium under paraffin oil at 37 °C in a humidified atmosphere of 5% CO_2 in air. Approximately 85% of the oocytes underwent GV breakdown within 3 h and, of these, approximately 80% subsequently emitted polar bodies. In the presence of db cyclic AMP (dbcAMP, 0.2 mM) <5% of the oocytes resumed meiosis, as shown by the retention of an intact GV and diffuse chromatin even after 16 h (refs 8 and 9). Air-dried chromosome spreads[11] of cultured oocytes confirmed that the oocytes were arrested in the dictyate stage of meiosis. *a*, *d*, Light microscopy of live oocytes cultured for 5 h with either dbcAMP (0.2 mM) (*a*) or dbcAMP (0.2 mM) and CCB (5 μg ml^{-1}) (*d*). Light microscopy was performed using a Zeiss Photomicroscope II equipped with Nomarski differential-interference optics (*a*, ×690; *d*, ×650). *b*, *c*, *e*, *f*, scanning electron microscopy of fixed oocytes cultured for 16 h with either dbcAMP (0.2 mM) (*b*, *c*) or dbcAMP (0.2 mM) and CCB (5 μg ml^{-1}) (*e*, *f*). Oocytes were fixed with 2% buffered glutaraldehyde and zona pellucidae were removed by brief treatment with pronase (0.5%). Oocytes were collected on polylysine-coated silver membrane filters (0.8 μm porosity, Flotronics) by gentle suction, post-fixed with 1% buffered OsO_4 for 30 min at 4 °C, washed with buffer, dehydrated through a graded series of acetones, and critical point dried out of liquid CO_2. Filters were mounted on aluminium studs, coated with gold and palladium in a sputtering device (Techniques Inc.), and examined in an ETEC Autoscan scanning electron microscope (*b*, ×690; *c*, ×5600; *e*, ×900; *f*, ×5400).

components, such as actin and tubulin, influence the surface properties of cells[11–13]. Sundqvist and Ehrnst[14] have reported that exposure of several types of cultured cells to CCB resulted in an asymmetrical redistribution of microfilaments and cell surface elements. Pseudocleavage may represent an analogous response to CCB by the oocyte.

It is interesting, in view of our results, that other investigators have observed a pronounced asymmetrical distribution of microvilli over individual blastomeres during early mammalian embryogenesis[15,16]. The changes in surface organisation seem to represent a very early morphogenetic event in the embryo. CCB-induced pseudocleavage may represent an aberrant induction of events that would normally occur later in development.

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Malignant transformation of rat embryo fibroblasts by herpes simplex virus types 1 and 2 at suboptimal temperature

HERPES SIMPLEX VIRUS (HSV) transforms cultured mammalian cells in three systems, involving (1) ultraviolet-irradiated virus[1–4], (2) increased incubation temperature[5,6] and (3) temperature-sensitive mutants[7,8]. Malignant transformation by HSV has been demonstrated for hamster, rat and mouse cells, using three systems[4,8–12]. We now report a further system, involving the use of a suboptimal temperature. This method is relatively convenient and seems to be applicable to various rat cell lines.

Sprague-Dawley rat single embryo cells (REF-1-76) were derived from a single clone, and grown as described before[12,13]. The cells were fibroblasts as shown in Fig. 1*a*. The normal REF-1-76 cells were established and propagated in tissue culture and so far they have not revealed any variability in the tests used in our laboratory in various tissue culture passages, even individual ones.

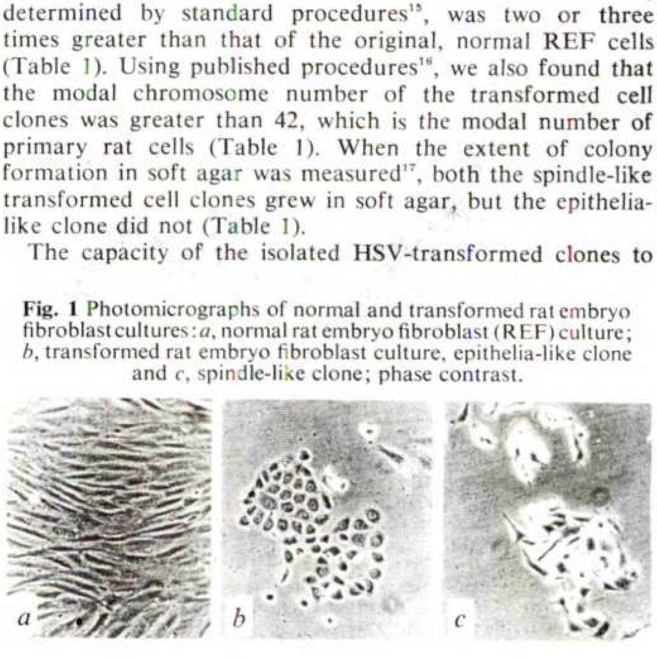
Three HSV strains, HSV type 1-Thea, HSV type 2-Müller and HSV type 2-HG-52, were propagated on human fibroblast cells and titred by a plaque assay[21,14]. The REF monolayer cultures in the 5th tissue culture passage were infected with HSV-1 or 2 at a multiplicity of infection (MOI) of 5 plaque-forming units (PFU) per cell. After 1 h of incubation at 37 °C the infected cells were shifted down to a suboptimal temperature of 20 °C. The cells were incubated for 1 h at 37 °C, washed five times with Hanks' solution, refed with conditioned medium[12] and divided into two parts. One part was incubated at 37 °C and the second part was shifted down to a suboptimal incubation temperature of 20 °C. Parallel cultures were mock-infected and incubated in the same way as the infected cultures and served as a control. Infected cells developed a typically HSV-induced cytopathic effect (CPE) and were destroyed when incubated at 37 °C.

Ninety per cent of the cells survived this incubation at 20 °C. The floating cells were collected by low-speed centrifugation (800g at 4 °C for 10 min), and transferred by trypsinisation to cell culture bottles. The appearance of floating cells seems to have been due to proliferating cells, which after treatment with 0.25% trypsin at 25 °C and seeding for the next passage in tissue culture lost their floating property and grew in monolayers. The monolayers and floating cells were then shifted separately up to 37 °C and maintained at this temperature. Two weeks later new cell colony formation was observed in the case of the floating cells which had been seeded and subsequently incubated at 37 °C. Transformation efficiency was approximately one colony per 10^5 original floating cells, corresponding to the formation of one colony per 1×10^6 HSV-infected rat embryo fibroblast cells. In contrast, the monolayer cells developed a typically HSV CPE and completely degenerated.

Mock-infected control cultures survived our procedure without forming foci and without producing floating cells. The new cell colonies were morphologically different from each other and different from the control cultures (Table 1). The new cells were either epithelia-like (Fig. 1*b*) or spindle-like (Fig. 1*c*), as reported for HSV-transformed REF cells[7,12]. Some clones were isolated by being picked from foci of colonies which had originated from floating cells and become established in tissue culture. As Table 1 shows, HSV-1-Thea was used for transformation of the isolated cell clone REF-T-20; HSV-2-Müller was used for REF-T-20-2, and HSV-2-HG-52 was used for REF-T-20-3. Two of the isolated HSV-transformed clones formed infectious virus after three to five subpassages and then lysed. The other isolated clones were stable in tissue culture and did not produce infectious HSV (clones 1, 2 and 3 in Table 1). The plating efficiency of normal and transformed cell clones, determined by standard procedures[15], was two or three times greater than that of the original, normal REF cells (Table 1). Using published procedures[16], we also found that the modal chromosome number of the transformed cell clones was greater than 42, which is the modal number of primary rat cells (Table 1). When the extent of colony formation in soft agar was measured[17], both the spindle-like transformed cell clones grew in soft agar, but the epithelia-like clone did not (Table 1).

The capacity of the isolated HSV-transformed clones to

Fig. 1 Photomicrographs of normal and transformed rat embryo fibroblast cultures: *a*, normal rat embryo fibroblast (REF) culture; *b*, transformed rat embryo fibroblast culture, epithelia-like clone and *c*, spindle-like clone; phase contrast.



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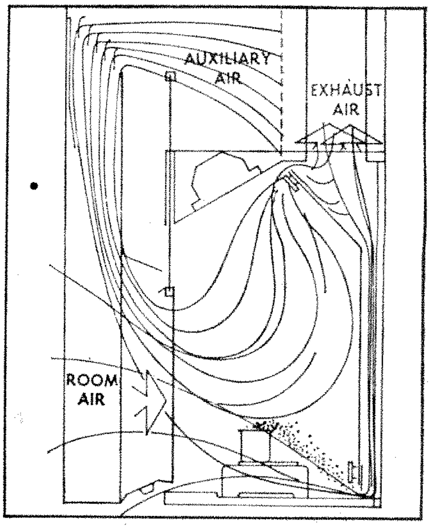

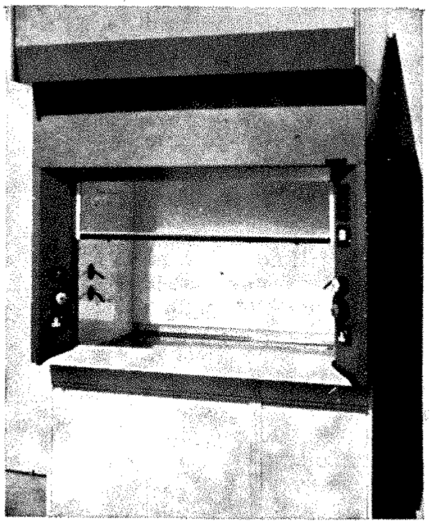

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Table 1 Properties of isolated HSV-transformed rat embryo fibroblast cells at suboptimal temperature (20 °C)

Cell types and number of clones	Virus	Morphology of cells	Plating Efficiency of cells %	Modal no. of chromosomes	Growth in soft agar	Tumour induction No. of cells inoculated	Tumour induction Efficiency
REF normal	Mock	Fibroblast-like	20	42	—	1×10^8	0/10*
REF-T-20-1	HSV-1-Thea	Spindle-like	58	89	+	1×10^6	10/10
REF-T-20-2	HSV-2-Müller	Spindle-like	52	97	+	1×10^6	6/6
REF-T-20-3	HSV-2-HG-52	Epithelia-like	40	66	—	1×10^7	0/10

*No. of tumours per no. of animals inoculated.

induce tumours in rats was evaluated. Newborn Sprague–Dawley rats (a closed but not strictly inbred colony), 1–2 d old, were inoculated with either normal or transformed REF cells, using 1×10^6 to 1×10^8 cells per rat. Animals were observed at least twice weekly for the development of palpable tumours at the site of inoculation. Table 1 shows that the two spindle-like transformed cell lines were highly tumorigenic in syngeneic animals. The induced tumours grew very rapidly and induced metastases in the lung. Histopathologically, the tumours were immature spindle-cell sarcomas. No regression of tumour growth was observed. The tumours could be transplanted successfully directly from animal to animal, or could be explanted and grown in tissue culture. Such cell cultures had the same morphological characteristics as the cells originally inoculated.

The detection of HSV antigens in the isolated cell clones and cell lines derived from the tumours was investigated by the indirect method of immunofluorescence, using hyperimmune rabbit antisera. The *in vitro* transformed cell clones with spindle-like morphology showed cytoplasmic HSV immunofluorescence. In contrast, the epithelia-like cell clone showed very weak fluorescence (Table 2). Cells derived from the tumours also exhibited HSV cytoplasmic immunofluorescence comparable with that of the original *in vitro* transformed cells. The isolated transformed cell clones were checked for resistance against HSV-superinfection. Monolayers of 5×10^5 cells from each clone were infected with 1×10^2, 1×10^3 and 1×10^4 PFU of HSV-1-Thea or HSV-2-Muller and overlaid with conditioned medium containing 0.5% carboxylmethyl-cellulose as described previously[13,14]. After 3 d of incubation at 37 °C in 5% CO_2 the cultures were fixed with 5% formaldehyde in phosphate-buffered saline and stained with 1% crystal violet and virus plaques were counted (Table 2). The spindle-like transformed cells, in contrast to normal and epithelia-like transformed cells, were completely resistant to superinfection with HSV types 1 and 2 in these conditions. The superinfectability of the epithalial cell clone was considerably reduced, compared with REF cells, usually by a factor 10 against HSV-1 and 10^2 against HSV-2.

Table 2 Detection of HSV-antigens in HSV-transformed and normal rat embryo fibroblast cells and sensitivity to superinfection with HSV types 1 and 2

	Immunofluorescence		HSV-Inoculum					
			HSV-1 (Thea) [PFU per dish]*			HSV-2 (Muller) [PFU per dish]		
Cell types	Cytoplasmic	Nucleic	1×10^2	1×10^3	1×10^4	1×10^2	1×10^3	1×10^4
			No. of plaques per dish			No. of plaques per dish		
REF control	—	—	109	TNC	total CPE	103.5	TNC	total CPE
REF-T-20-1	+	—	—	—	—	—	—	—
REF-T-20-2	+	—	—	—	—	—	—	—
REF-T-20-3	(+)	—	24	196	TNC	1	15	153

TNC, too numerous to count.
*Petri dishes contained 5×10^5 cells.

RNA-directed DNA polymerase[15,16] was assayed to investigate the possible presence of oncorna viruses in the transformed cell clones, the tumour material and cell lines derived from the tumours (manuscript in preparation). The standard mixed culture cytopathogenicity test with XC cells[17] showed that in all cases, including cell lines derived from rat tumour biopsies, C-type virus particles were not detectable. Transformation at suboptimal temperature was repeated with two independently isolated rat embryo fibroblast cell cultures in three independent experiments and gave similar results in all cases.

In conclusion, we have shown that rat embryo fibroblasts inoculated with HSV-1 or 2 were transformed when the cells had been incubated at the suboptimal temperature of 20 °C for the first 3 weeks after infection. The transformed cell clones with spindle-like morphology were highly tumorigenic. In all control experiments the mock-infected cultures showed 20% plating efficiency and the modal chromosome number was 42. The cultures did not grow in soft agar and did not induce tumours in syngeneic animals. The mechanism of transformation at suboptimal temperature remains unclear and it is open to question whether or not the presence of part of the HSV genome is required for the maintenance of the transformed state.

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Secretion of hCG-α subunit and hCG by HeLa strains

IN recent letters[1,2], we have independently reported seemingly different secretion patterns of human chorionic gonadotropin (hCG) and its alpha (hCG-α) and beta (hCG-β) subunits by certain HeLa strains. These letters were further discussed in an article in News and Views[3]. Ghosh and Cox[1] found that HeLa 65 and HeLa 71 secreted a product with activity in an 'hCG-β' immunoassay (detecting hCG and/or hCG-β), and that this secretion was greatly stimulated by butyrate; hCG-α was not measured. Lieblich *et al.*[2] reported that HeLa CCL 2, 2.1 and 2.2 secreted very large quantities of hCG-α but no detectable amounts of hCG or hCG-β (<0.2 ng ml^{-1}). Two questions were raised: what was the nature of the immunoactive material in the hCG-β assay, and could the differences between the two laboratories be attributed to the known variability among HeLa strains? We have collaborated in an attempt to answer these questions, and we confirmed strain differences in hCG-α secretion in HeLa cells.

HeLa 65 and HeLa 71 were grown in increasing concentrations of sodium butyrate at New York University, and the media were analysed for hCG, hCG-β and hCG-α at the National Institutes of Health. In separate, specific radioimmunoassays (RIA), we have identified the 'hCG-β' immunoactivity as complete hCG; no free hCG-β was found (<1 ng ml^{-1}). Both HeLa strains secreted much larger quantities of free hCG-α than complete hCG (Table 1), but not sufficient hCG-α to produce significant cross reaction in the hCG RIA. Sodium butyrate stimulated both HeLa strains to increase markedly the secretion of both proteins, with the secretion of hCG-α far in excess of hCG. Although the relative stimulation by butyrate of hCG and hCG-α were within an order of magnitude (~100–500-fold), the absolute levels of hCG-α secretion exceeded complete hCG by 15-fold in HeLa 65 and 500-fold in HeLa 71. These differences between HeLa 65 and HeLa 71 in basal and butyrate-stimulated secretion of hCG and hCG-α, and the previously reported[2] differences among HeLa 2, 2.1 and 2.2 in basal hCG-α secretion may be functional counterparts of the well-described karyotypic differences among strains of HeLa.

Table 1 Basal and butyrate-stimulated secretion* of hCG-α subunit and hCG in two HeLa strains

	Butyrate concentration (mM)	Cell no. ($\times 10^{-6}$)	hCG (pmol per 10^6 cells per day)	hCG-α (pmol per 10^6 cells per day)
HeLa 65	0	17	0.01	<0.03
	1	11	0.6	2.0
	5	2.2	1.1	16
HeLa 71	0	22	0.02	15
	1	8.2	0.4	430
	5	0.3	3.0	1,450

*HeLa cells grown for 3 days in Waymouth's medium containing 10% foetal calf serum with additions of sodium butyrate as shown.

We have tested butyrate stimulation of another cell line producing α subunit ectopically. Preliminary studies in ChaGo (ref. 4), derived from a primary carcinoma of the lung, also show significant butyrate stimulation of hCG-α secretion rates. Demonstration of butyrate stimulation in a second ectopic protein-producing cell line underlines the potential of this reagent in the study of biosynthetic mechanisms.

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Effects of immunisation against luteinising hormone releasing hormone on reproduction of the marmoset monkey *Callithrix jacchus*

LUTEINISING hormone releasing hormone (LHRH) is a decapeptide secreted by the hypothalamus and transported through the hypophysial portal system to the anterior pituitary gland where it acts to stimulate gonadotrophin release[1]. Selective neutralisation of this releasing factor by specific antibodies has been achieved in rodents[2–4], but there has been no report of similar work on primates. Immunisation against hypothalamic releasing hormones is a potentially suitable method for selective control of the secretion of anterior pituitary hormones. In particular, interference with LHRH release might make possible a new approach to contraception. We describe here how antibodies to LHRH were raised in the marmoset, *Callithrix jacchus*, and outline the subsequent effects on gonadal activity and pituitary function.

Ten adult marmosets, five male and five female, were immunised with synthetic LHRH conjugated to bovine serum albumin (BSA) using the carbodiimide technique[5]. Each animal received eight intradermal injections of the conjugate emulsified in Freund's adjuvant (FCA), followed by *Bordetella pertussis* vaccine as an additional adjuvant. Four further animals, two male and two female, were injected with BSA alone in FCA, together with *B. pertussis* vaccine, and served as controls. Each animal received a booster injection approximately 10 weeks after primary immunisation.

Heparinised blood samples were collected once a week from the femoral vein, centrifuged immediately and plasma was stored at −20 °C for subsequent determination of hormone and antibody levels. Testicular and uterine dimensions were also measured once weekly, using calipers. Progesterone, LH, and testosterone were measured by radioimmunoassay techniques described elsewhere[6–8]. The presence of antibody to LHRH was assessed by measuring the percentage binding of approximately 10 pg radio-iodinated LHRH by plasma at a dilution of 1 : 500.

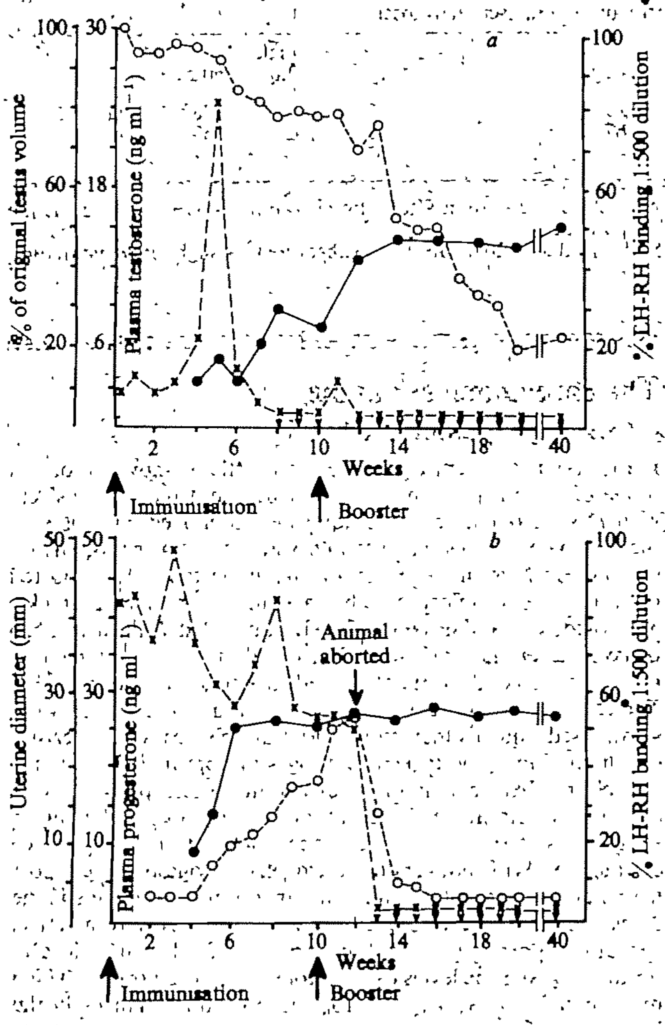

Fig. 1 Effects of immunisation against LHRH in the marmoset. *a*, Testosterone concentration (×), testis volume (○), and antibody titre (●) in a male; *b*, progesterone concentration (×), uterine diameter (○) and antibody titre (●) in a female. Arrows indicate undetectable.

No antibodies to LHRH were produced by the control animals and their gonadal activity was normal. Although all LH-RH-immunised animals produced antibodies, in four cases the titres were low (< 15% binding).

Of the six animals which developed high antibody titres (47–57% binding) all three males showed marked testicular atrophy and suppression of testosterone secretion, while all females developed inactive ovaries and became acyclic. Figure 1 illustrates the effects of immunisation against LHRH in one male and one female marmoset. In the male (Fig. 1*a*) antibody titres increased gradually during the first 10 weeks, but plasma testosterone levels were not depressed until after the booster injection, when the concentration of antibodies increased markedly. Plasma testosterone then decreased to undetectable levels (< 1 ng ml^{-1}) and the testes became softer and smaller, eventually shrinking to less than 25% of their original volume.

The female (Fig. 1*b*) was about 2 weeks pregnant at the time of immunisation and showed a more rapid build up of antibody titres during the following 6 weeks, while pregnancy continued normally. The antibody titre had reached a maximum by week 8 and the booster produced no further increase. Progesterone then began to decrease, dropping rapidly at week 12 to undetectable levels as the animal aborted. Progesterone concentrations were minimal (< 5 ng ml^{-1}) for up to 40 weeks after immunisation, showing that ovulation had ceased.

Basal LH levels in normal marmosets fluctuate around the limit of sensitivity of the LH assay (20 ng ml^{-1} LHRP-1 equivalent). Consequently, it was not possible to detect any decrease in LH concentrations after immunisation, and these results have been omitted from this report.

Histological sections of the control testes showed normal spermatogenesis, but in the three males with high levels of antibody (52–55% binding) there was marked involution of the seminiferous tubules, and spermatogenesis was severely impaired (Table 1). Interstitial cells were greatly reduced in size and contained condensed nuclei. Although seminiferous tubules in animals with low antibody titres (< 15% binding) were also reduced in size, they were considerably larger than the tubules in animals with high antibody titres, and spermatogenesis had not been affected. Ovaries in female marmosets with high antibody titres (47–57% binding) were much reduced in size, and although follicular development could be seen there was no luteal tissue. Ovaries in females with low antibody titres (< 15% binding) were not significantly reduced in size, and contained both follicular and luteal tissue (Table 1).

The effect of immunisation on the response of the pituitary to exogenous LHRH was also studied. Plasma LH levels were measured immediately before, and 45 min after intramuscular injections of various doses of LHRH (4 μg, 10 μg and 50 μg) to animals 40 weeks after immunisation (Table 2). Animals with high antibody titres showed a much reduced response at all doses compared with animals with low antibody titres, and with controls. A dose response relationship was demonstrated by high titre animals, whereas animals with low antibody titres showed a maximal response to all three doses of LHRH Even in animals with high antibody titres that had been immunised for 40 weeks and were infertile, the pituitary gonadotrophs were still able to respond to the releasing hormone. An LHRH test on immunised animals may provide a better

Table 1 Ovarian and seminiferous tubule measurements in control and immunised marmosets

Group	Tubule diameter (μm) Mean ± s.d.	Ovarian size length (mm) × breadth (mm) Mean ± s.d.
Control	279.5±21.7	61.4±2.2
LHRH-immunised high titres	132.2±9.9	12.4±1.7
LHRH-immunised low titres	245.1±21.1	47.6±3.9

In each group 20 tubule diameters from two males were measured. The mean tubular diameter in each group of immunised animals was significantly smaller than that in control animals ($P < 0.001$; Student's t test). Laparotomy was performed on two females in each group and the right ovaries were measured across their longest and shortest axes. The product of these two measurements was taken as a rough indication of ovarian size. Ovaries in animals with high antibody titres were significantly smaller than those in the controls ($P < 0.001$; Student's t test). There was no significant difference in ovarian size between control animals and animals with low antibody titres.

Table 2 Response to LHRH in control and immunised marmosets

	Male			Female		
	4μg	10μg	50μg	4μg	10μg	50μg
LHRH-immunised high titre (6)	0†	14	23*	7*	22	28*
LHRH-immunised low titre (4)	79	63*	67	72	68*	63*
Control (2)	83	—	—	87*	—	—

Plasma LH concentrations (ng ml^{-1} LHRP-1 equivalent) were measured before and 45 min after administration of LHRH, and the net increase (Δ LH) is shown. The total number of animals used is indicated in parentheses.

* Pre-injection levels were undetectable (< 20 ng ml^{-1}) and to calculate Δ LH, a value of 20 ng ml^{-1} was substituted. A slight underestimation of Δ LH values has therefore resulted.

† Both pre- and post-injection levels were undetectable.

indication of the effectiveness of immunisation than measurement of antibody titres.

These results demonstrate that immunisation against LHRH is an effective way of suppressing gonadal activity in the marmoset. Although the necessity for Freund's adjuvant and the rather drastic effects of complete inhibition of LHRH preclude its acceptability as a method of human fertility control at the moment, it may still be possible to develop a new approach to contraception by LHRH inhibition, achieved either immunologically, or by the use of competitive LHRH antagonists Total suppression of the testis would be necessary in the male to ensure infertility, and this would probably be undesirable In the female, however, it may be possible to suppress the ovary to a level which would effectively prevent ovulation, but would still allow some follicular development and oestrogen secretion.

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The carcinogen ethionine elevates progesterone levels

ETHIONINE is a potent carcinogen, but little is known about its mode of action. It is known that it does not react extensively with DNA[1]. It may be an ubiquitous carcinogen, as it is synthesised by *Escherichia coli*[2] and several other microorganisms, so studies of its mode of action are important. We reported recently a novel effect of ethionine in the immature chick[3]. The oviducts of chicks previously stimulated with oestrogen behaved after the administration of ethionine as if they had received a second stimulation with oestrogen or progestin[4-6]. The egg white proteins, ovalbumin and conalbumin were synthesised and distinct morphological changes, characteristic of stimulation by oestrogen or progestin appeared. It was thus not clear whether ethionine (or its metabolic product) was affecting steroid hormone receptors[7] directly, or interferring in some way with steroid hormone metabolism to produce elevated levels of endogenous oestrogen or progestin. We report here that ethionine or its sulphoxide are unique in inducing in the chick oviduct the effects cited above; analogues were inactive. We also report that the administration of ethionine elevates serum progesterone levels over 10-fold, to a concentration which may be sufficient to account for the profound changes in the immature oviduct.

We used the procedure developed in the laboratories of O'Malley[8,9] and Schimke[4,5,10]. Oestrogen administration to the immature chicks results in extensive cytodifferentiation of the primitive oviduct and synthesis of cell-specific proteins. Ovalbumin synthesis is first detected approximately 18 h after oestrogen stimulation, and after 7–10 d of daily stimulation it accounts for 50–60% of total protein synthesis (primary stimulation)[11]. Discontinuation of oestrogen administration is accompanied by a gradual loss in cell-specific protein synthesis. Ovalbumin synthesis is not detectable after 15–20 d of oestrogen

Table 1 Effect of analogues and metabolites of ethionine on induction of ovalbumin synthesis in immature chick oviduct

Treatment	^{14}C-amino acids incorporated, per mg soluble protein (c p.m. × 10^{-4})	^{14}C-amino acids incorporated into ovalbumin, per mg soluble protein (c.p.m. × 10^{-4})	Ovalbumin synthesis, % of total protein synthesis
None	1.84	ND	0
L-Ethionine	2.43	0.59	24.3
L-Ethionine sulphoxide	1.91	0.327	17
	1.57	0 062	4
L-Ethionine sulphone	1.03	ND	0
	1.19	ND	0
N-Acetyl-L-ethionine	1.42	ND	0
	1.47	ND	0
S-Propylhomocysteine	1.03	ND	0
	1.46	ND	0

Four day old chicks were injected with 1 mg of oestradiol 17-β-benzoate in sesame oil for 10 d. After a withdrawal period of 4 weeks, the chicks were injected (in groups of eight), intraperitoneally (i.p) with a 1-ml suspension, in water, of L-ethionine or its analogue and metabolites (0.25 mg per g body weight) and adenine (0 12 mg per g body weight) for 5 d. On the sixth day the animals were sacrificed, the oviducts removed, the magnum portions of the oviduct freed of adjoining tissue and cut into small pieces. The magnum explants were incubated *in vitro* in antibiotic free medium containing labelled amino acids[13] Tissue was incubated in 2 ml of medium in 25-ml Erlenmeyer flasks for 3 h. Each flask contained tissue from three oviduct magna. Ovalbumin synthesis in the magnum explant was determined by specific immunoprecipitation as described before[14-16]. L-ethionine was from Sigma, L-ethionine sulphoxide, L-ethionine sulphone and *N*-acetyl-L-ethionine were from Cyclo. *S*-propylhomocysteine was synthesised in our laboratory[17]. ND, not detected.

Table 2 Elevation of progesterone levels in immature chicks by ethionine

Treatment	Ovalbumin synthesis in oviducts, % of total protein synthesis	Serum progesterone (ng per 100 ml)	Serum 17-β oestradiol (ng per 100 ml)	Total serum cholesterol (mg per 100 ml)
None	ND	29	10	103
Ethionine + Adenine				
3 d	ND	40	8	155
4 d	6	153	7	150
5 d	16	285	3	114
Oestradiol 17-β benzoate, 2 d	35	40	448	361
Progesterone, 2 d	34	523	7	113

After 10 d of primary oestrogen stimulation chicks were withdrawn for 21 d and (in groups of eight) injected i.p. with ethionine and adenine as described for Table 1. Oestradiol or progesterone (2 mg per chick, in sesame oil) were injected intramuscularly. At the times specified, blood was collected by cardiac puncture and the separated serum was stored frozen until analysed. Analyses for progesterone[18], 17-β-oestradiol[19] and total cholesterol[20] in serum were performed by Laboratory Procedures, Upjohn. The values are the average of duplicate determinations. These experiments were repeated with another group of chicks with essentially the same results (data not shown). Ovalbumin synthesis was determined in duplicate and the values are an average. There were some variations in the time course and degree of induction of ovalbumin synthesis and progesterone by ethionine.

withdrawal (withdrawn chicks). Administration of oestrogen or progesterone[4,5] or ethionine[3] to withdrawn chicks results in rapid restoration of cell-specific protein synthesis in the chick oviduct.

To ascertain whether ethionine analogues or its metabolites had similar biological activity, we tested several related compounds. There was a rigid structural requirement for ethionine in the induction of ovalbumin synthesis. Ovalbumin synthesis produced by ethionine sulphoxide was less extensive than by ethionine (Table 1). In the rat, ethionine is metabolised to ethionine sulphoxide, *N*-acetylethionine and *N*-acetylethionine sulphoxide, but ethionine sulphoxide and *N*-acetylethionine can be converted back to ethionine[17]. In the chick the metabolism of *N*-acetylethionine seemed to be different, as it failed to induce ovalbumin synthesis. The sulphone derivative of ethionine and its propyl homologue failed to elicit the synthesis of ovalbumin.

The administration of ethionine plus adenine produced an elevation of up to 10-fold in serum progesterone levels but had no significant effect on the levels of 17-β-oestradiol (Table 2). Progesterone, unlike oestrogen, is incapable of inducing cytodifferentiation of tubular gland cells or ovalbumin synthesis in the unstimulated immature chick oviduct[4–6]. Ethionine and progesterone are similar in this respect; they produced no pronounced effects on specific protein synthesis without previous oestrogen stimulation[3]. Furthermore, there is little growth of the oviduct during secondary stimulation with ethionine, another characteristic of the administration of progestins.

Since these determinations were done by radioimmunoassay by a commercial firm (see legend to Table 2), we determined the reproducibility of the individual values. Serum samples were re-submitted for analysis after storage for 1 month at −15 °C, under different code numbers. In no case was the variation greater than 2-fold whereas the increment in progesterone level was more than 10-fold. Given the complexity of the assay and the unknown effects of storage, the values obtained may be considered valid.

The mechanism of the elevation of serum progesterone levels by ethionine is obscure, it could be the result of increased synthesis of progesterone or decreased degradation or excretion.

Cholesterol was also determined in the serum of chicks (Table 2). Increase in cholesterol synthesis in the rat is induced by the administration of several carcinogens, such as ethionine, aflatoxin, *N*-2-fluorenylacetamide and 3-methyl-4-dimethyl aminoazobenzene[21]. The effect is thought to be a loss of feedback control of synthesis by dietary cholesterol. These changes in cholesterol synthesis are observed much before the appearance of any pathological stigmata of tumours. Increased rate of cholesterol synthesis and impairment of its feedback control is also characteristic of a variety of primary and transplantable tumours[21] and lymphocytic leukaemic cells[22]. Whether the elevation in the levels of cholesterol which is a precursor of steroid hormones is causal in the elevated levels of progesterone is not known.

Elevation of the progesterone levels by the carcinogen ethionine may have far reaching implications. The importance of endocrine activity in neoplasia is well known[23]. Whether this novel effect of ethionine is unique among chemical carcinogens in producing an imbalance in hormone metabolism remains to be determined.

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Sodium nitroprusside and other smooth muscle-relaxants increase cyclic GMP levels in rat ductus deferens

SMOOTH muscle tone seems to be primarily regulated by the concentration of free calcium in cytoplasm[1,2]. Several agents that cause smooth muscle contraction increase the tissue content of cyclic GMP with no significant change or only small reduction of the cyclic AMP concentration[3–7]. It has been suggested[5–7] that cyclic GMP may be casually involved in the contractile response of smooth muscle and that this nucleotide may act as a comediator with calcium to promote contraction. Several observations, however, are not consistent with this assumption. Although increases both in tissue tonus[1,2] and in the cyclic GMP level[8–10] induced by hormones and neurotransmitters are generally dependent on the presence of extracellular calcium and seem to be secondary to increased influx of calcium into the cytoplasm, the correlation between these two calcium-dependent events is poor in various tissues[10–16]. On the basis of such observations, we have suggested that cyclic GMP may act as a negative feedback inhibitor of hormonally stimulated calcium influx into cytoplasm[8,10,11]. We have studied the effects of various agents on cyclic nucleotide levels in the ductus deferens of the rat, and report here that many smooth muscle relaxants, including sodium nitroprusside (SNP), increase cyclic GMP levels in the ductus deferens.

Although several of the tested agents are more potent in vascular smooth muscle than in tissues of the visceral type of smooth muscle, the ductus deferens was used, since in this tissue the contribution of smooth muscle cells to tissue mass is higher than in most other smooth muscular tissues and the importance of extracellular calcium for hormone effects on cyclic GMP levels is well established[8–16].

SNP is a potent vasodilator[17] and causes relaxation of the potassium-depolarised ductus deferens (K.D.S., unpublished). Addition of SNP to segments of ductus deferens increased the cyclic GMP level severalfold (Table 1). When calcium was omitted from the incubation medium, basal cyclic GMP levels were decreased and the effect of acetylcholine on the cyclic GMP level was abolished, whereas the effect of SNP was still observed. In the absence of extracellular calcium, SNP (0.1 mM) increased the cyclic GMP level about 50-fold within 20 s, and a maximal increase of about 100-fold was observed after 1 min (Fig. 1). Half-maximal stimulation was caused by 10 μM SNP, and the effect on the cyclic GMP level was maximally developed with about 1 mM SNP (see Fig. 1). Cyclic AMP levels were not changed in any of these conditions.

Hydroxylamine increases the cyclic GMP level in rat cerebral cortex[20] and caused relaxation of various smooth muscular tissues including ductus deferens (K.D.S., unpublished). In our system, hydroxylamine 0.1 mM increased the cyclic GMP level of the ductus deferens 4-fold, and 20- to 30-fold in the presence and absence of extracellular calcium, respectively; cyclic AMP levels were unchanged (Table 2).

Various other smooth muscle-relaxants and antihypertensives also increased cyclic GMP levels in the rat ductus deferens when they were added in the absence of extracellular calcium (Table 3). Nitroglycerol (0.1 mM) and sodium nitrite (1 mM), agents that have been shown to increase the cyclic GMP content in other tissues[13,19,21,22], increased the cyclic GMP level of the ductus deferens about 2-fold after 3 min Furthermore, the antihypertensives diazoxide, hydralazine and minoxidil; the coronary

Table 1 Influence of extracellular Ca^{2+} on the effects of SNP and acetylcholine on rat ductus deferens cyclic GMP level

Addition	Cyclic GMP concentration (pmol per g wet weight): 2 mM Ca^{2+}	no Ca^{2+}
None (control)	56.4±5.6 (13)	10.6±1.5 (15)
Acetylcholine	163±22.6 (9)	12.7±2.1 (10)
SNP	585±79 (7)	596±39 (7)

Tissue segments were preincubated for 30 min in a balanced salt solution[18] containing 30 μM EDTA and 2 mM or no calcium chloride. Tissue was then transferred to fresh medium containing 2 mM or no Ca^{2+} as above with or without addition of 0.1 mM acetylcholine or 0.1 mM sodium nitroprusside. After 5 min of incubation, tissue was fixed, and cyclic nucleotides were extracted, purified and assayed by established methods[19]. Data are mean ±s.e.m., with the number of samples in parentheses. Cyclic AMP levels were not significantly changed in any of the above conditions.

Fig. 1 Effect of SNP on cyclic nucleotide levels in the ductus deferens. Segments of tissue were preincubated for 30 min in medium without addition of calcium. Sodium nitroprusside was then added for 3 min at the concentrations indicated on the abscissa in *a*. A time course of the response was obtained by adding 0.1 mM sodium nitroprusside for the times indicated on the abscissa in *b*. Data are mean ± s.e.m., the number of tissue samples per group was 7.

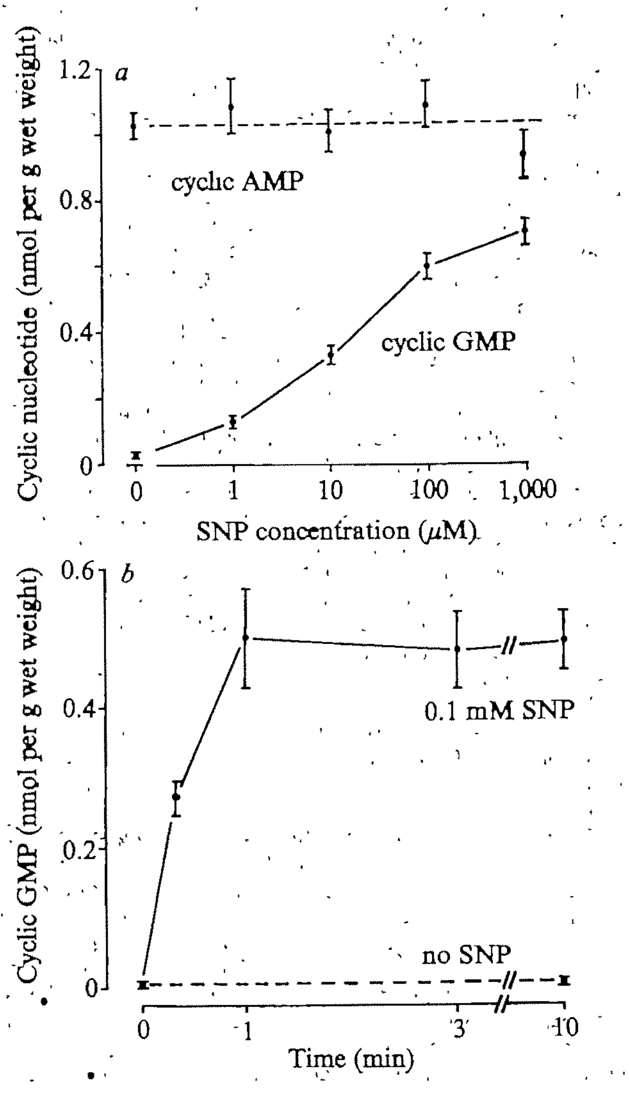

Table 2 Effect of hydroxylamine on cyclic nucleotide levels in presence and absence of Ca^{2+}

	Concentration (pmol per g wet weight) Cyclic GMP	Cyclic AMP
2 mM Ca^{2+} Control	42.8 ± 2.9 (7)	914 ± 34 (8)
Plus hydroxylamine (5 min)	177.7 ± 25.5 (8)	1002 ± 91 (8)
no Ca^{2+} Control	9.5 ± 2.2 (8)	1051 ± 88 (8)
Plus hydroxylamine (5 min)	195.4 ± 19.7 (8)	1067 ± 71 (8)
Plus hydroxylamine (10 min)	260.3 ± 25.4 (7)	970 ± 37 (5)

Segments of rat ductus deferens were preincubated for 30 min without addition of calcium. After 5 min incubation with or without 2 mM calcium, hydroxylamine (0.1 mM) was added for 5 to 10 min. Control values were not significantly changed over this period. Values are mean ± s.e.m.

artery dilators dipyridamole, prenylamine, cinnarizine, lidoflazine, perhexiline, and the methoxy derivative of verapamil (D-600), SKF-525A and chlorpromazine (0.1 mM each) each caused a 2- to 3-fold increase in the cyclic GMP level when added for 3 min to segments of ductus deferens. In contrast, the local anesthetic tetracaine, phenytoin and pentobarbital (0.1 mM each) did not change the cyclic GMP significantly (Table 3), although these agents caused relaxation of the K^+-depolarised tissue. Cyclic AMP levels were not affected by any of the above compounds.

The mechanism by which these agents increase the cyclic GMP level independently of the presence of calcium is not known. Increased cyclic GMP formation is more likely than reduced degradation of the nucleotide, as the effect of the potent phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine, on the cyclic GMP level in the ductus deferens depended on the presence of calcium whereas the effect on the cyclic AMP level was not affected by calcium[8]. Although several of the above drugs are capable of inhibiting phosphodiesterases in cell-free sytems[23], none of the agents changed the cyclic AMP level in the ductus deferens. Furthermore, SNP, sodium nitrite and hydroxylamine all stimulate guanylate cyclase from rat ductus deferens (H. Graf and G.S., in preparation).

Table 3 Effect of smooth muscle relaxing drugs on cyclic nucleotide levels

(Experimental series I)	Concentration (pmol per g wet weight) Cyclic GMP	Cyclic AMP
Control	24.6 ± 4.2 (23)	1052 ± 35 (25)
Ethanol	29.2 ± 7.5 (14)	1006 ± 59 (15)
Nitroglycerol	65.7 ± 6.9 (14)	1061 ± 61 (13)
Sodium nitrite	65.1 ± 7.0 (11)	1001 ± 81 (5)
D-600	59.9 ± 6.2 (14)	955 ± 91 (8)
Cinnarizine	67.4 ± 14.0 (8)	977 ± 91 (10)
Lidoflazine	68.6 ± 17.6 (11)	1020 ± 78 (11)
Perhexiline	55.2 ± 14.4 (8)	922 ± 76 (11)
Minoxidil	84.2 ± 17.9 (8)	996 ± 64 (12)
Hydralazine	61.9 ± 15.7 (10)	1054 ± 65 (13)
Diazoxide	57.4 ± 8.3 (10)	1128 ± 69 (11)
(Experimental series II)		
Control	5.8 ± 1.1 (8)	1025 ± 25 (7)
Prenylamine	28.9 ± 9.5 (7)	954 ± 31 (8)
Dipyridamole	36.3 ± 4.4 (7)	972 ± 50 (6)
Chlorpromazine	29.1 ± 9.3 (9)	942 ± 49 (8)
SKF-525 A	22.8 ± 4.3 (8)	896 ± 33 (7)
Tetracaine	7.7 ± 1.5 (9)	970 ± 61 (9)
Phenytoin	8.3 ± 3.3 (9)	923 ± 36 (9)
Pentobarbital	6.7 ± 2.4 (7)	958 ± 41 (7)

Segments of ductus deferens were preincubated for 30 min in buffer without addition of calcium. Drugs were then added for 3 min at a concentration of 0.1 mM and sodium nitrite at 1 mM. Ethanol (0.2%, v/v) was used as a solvent of nitroglycerol and perhexiline. Values are mean ± s.e.m., with number of samples in parentheses.

D-600, prenylamine and several other of the active drugs are known to reduce the responses of smooth muscule and other tissues to hormonal stimuli by their so-called calcium-antagonistic property[24–26]. The 8-bromo derivative of cyclic GMP exogenously applied (10^{-5} M) also reduced the contractile responses of the rat ductus deferens and other tissues to some stimuli and diminished the effect of calcium on the tonus of the K^+-depolarised ductus deferens (K.D.S., V. Kreye and G.S.). These observations together with our finding that relaxing drugs as well as contraction-producing hormones increase cyclic GMP levels support the hypothesis[8,10,11] that cyclic GMP may reduce or prevent calcium influx into the cytoplasm of smooth muscle and possibly other cells and may thereby reduce hormone-induced excitation or excitability.

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Trypsin inhibits the action of tetrodotoxin on neurones

Evidence suggests that the molecules responsible for membrane excitability are proteins[1–4], but they have not been isolated in spite of considerable efforts and their identities remain unknown. Some clues have come from study of the effects of enzymes and group-specific chemicals on appropriate electrophysiological properties of the membrane[1–4]. In certain nerve and muscle cells the voltage-dependent inward currents which generate action potentials may be carried by Na^+ or Ca^{2+} (refs 5–13). Tetrodotoxin (TTX) specifically blocks the voltage-dependent inward Na^+ current in many of these cells[14], including neurones of *Aplysia*[10] and *Helix*[11] and is widely used for this reason. It also has the advantage of being a relatively simple molecule of known atomic structure[14]. But TTX has no effect on *Helix* neurones when they are isolated from the ganglia in which they are located[13]. The ganglia are first pretreated with trypsin and we wondered whether the lack of TTX-sensitivity in these preparations was due to some action of this enzyme on the TTX receptor. We have now found that exogenous trypsin modifies the TTX-binding properties of the Na channel without affecting its reversal potential or selectivity against Tris$^+$ and has no measurable effect on the Ca^{2+} channel. Because trypsin splits polypeptide chains on the carboxyl side of arginine or lysine residues[15], it seems likely that the TTX receptor contains either or both of these amino acids but that the selectivity site does not.

We have developed a simple suction pipette method with which individual nerve cell bodies can be isolated, perfused internally and voltage clamped. This method will be valuable in the study of individual cell membranes of excitable or inexcitable tissues.

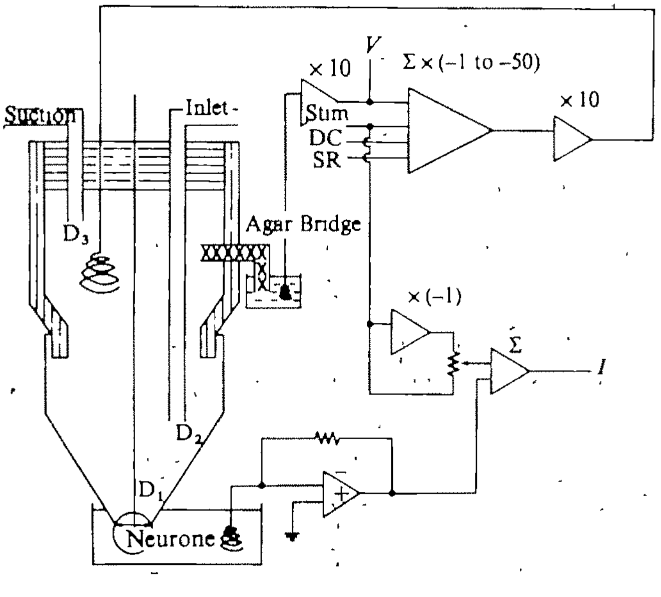

Fig. 1 Schematic of experimental setup. D$_1$ = 25 µm, D$_2$ = 500 µm, and D$_3$ = 2 mm SR, series resistance compensation. Σ is the summing amplifier used to offset the shunt resistance.

We examined neurones from the circumoesophageal ganglia of *Helix aspersa* using a suction pipette with an internal diameter of 15–30 µm (Fig. 1), filled with a solution of 50 mM K aspartate and 55 mM KH$_2$PO$_4$ adjusted to *p*H 7.4 with 28 mM Tris$^+$ base[16]. The extracellular solution bathing the neurones had the following composition: 75 mM NaCl; 5 mM KCl; 10 mM CaCl$_2$; 15 mM MgCl$_2$, and 5 mM Tris-HCl, *p*H 7.6 The neurones were exposed by stripping off the capsule of the ganglion with jeweller's forceps. A neurone with a diameter ranging from 80 to 250 µm was sucked into the tip of the pipette and isolated by rending it free from neighbouring neurones. A platinised platinum wire (5 µm tip) was then advanced into the neurone. It had a resistance of 3×10^4 Ω and was connected through an agar salt bridge and Ag AgCl wire to the voltage amplifier (Fig. 1) A reference for the voltage was also provided by a salt bridge connected to the bath, but this is not shown in Fig. 1. In these conditions and at a temperature of 18 °C we measured resting potentials of −60 mV. (after corrections for measured liquid junction potentials), input resistances of 15–20 MΩ, and action potentials with overshoots of 20–30 mV for periods averaging 6 h. Similar values were obtained when two 3 M KCl-filled micropipettes, one for passing current and the other for recording voltage, were inserted into the same neurone. These measurements indicate healthy neurones and we presume that the soma healed over at the site where it had been pulled free from its axon much the same way as cut cardiac muscle heals over.

The voltage clamp circuit included series resistance compensation[17] and a circuit for correcting the offset currents passing across the shunt resistance of 50 and 100 MΩ (ref. 18) that isolated the cell body from the extracellular solution (Fig. 1).

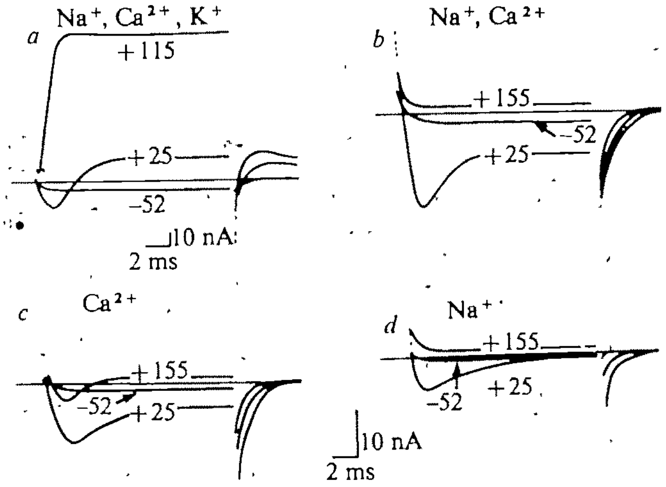

Fig. 2 Voltage clamp currents and their separation in *Helix* neurones. Holding potential, $V_H = -60$ mV. Voltage clamp potentials indicated above respective currents Note the differences in current calibration between (*a*) and (*b*), (*c*) and (*d*). *a*, Neurone perfused internally with control solution (see text). External solution contained CsCl. The voltage-dependent currents are due to Na$^+$, Ca^{2+} and K$^+$. The small depolarising step produces sustained inward current. The current tails are outward for the larger voltage steps. *b*, *c* and *d*, Current traces from a different neurone. *b*, After 20 min of internal perfusion with Cs aspartate, the voltage-dependent outward K$^+$ current is abolished. The remaining voltage-dependent currents are due to Na$^+$ and Ca^{2+}. Early current is outward at +155 mV and the persistent outward current is attributed to leakage. At +25 mV there is an early transient inward current and a later smaller persistent inward current. The tail currents are now inward at all steps and appear to recover more rapidly. *c*, Ten minutes after 3 × 10^{-6} M TTX was added to external solution Ca^{2+} current is separated Notice early inward current at +155 mV, indicating that E_{Ca} is more positive than this value Inward current at +25 mV is reduced by about 30% The Ca^{2+} current is abolished by verapamil or Co^{2+}. *d*, Same neurone as (*b*) and (*c*), 30 min after recovery from TTX and 5 min after addition of 5×10^{-6} M verapamil to the external solution. The Na$^+$ current is separated. The early current is outward at +155 mV, shows little inactivation at −52 mV, but inactivates almost completely at +25 mV. The Na$^+$ tail currents are smaller than the Ca^{2+} tail currents. The Na$^+$ current is blocked by TTX and substitution of Tris for Na$^+$ in the external solution.

The voltage clamp currents in the control solutions were elicited by voltage steps from holding potentials of −60 mV and had voltage-dependent Na$^+$, Ca^{2+} and K$^+$ components (Fig. 2*a*). They were separated from each other as follows. The K$^+$ current was blocked by substituting 110 mM Cs aspartate for the potassium salts in the control internal perfusion solution (Fig. 2*b*). We also substituted Cs$^+$ for K$^+$ in the extracellular solution. Addition of TEA to the internal or external media had no further effects. We used TTX (Sigma or Sankyo) to block the Na$^+$ current The action of TTX was dose-dependent and reversible TTX in doses of 3×10^{-7} M reduced the inward current by an average of 30% in 40 neurones (Fig 2*c*). Similar doses were also effective in *Aplysia* neurones[19]. Replacement of Na$^+$ in the external solution with Tris$^+$ had a similar effect to TTX in 20 neurones. Thus, 30% of the inward current was carried by Na^+. This Na^+ current was isolated after blocking Ca^{2+} current with verapamil (Knoll) in doses of $1–5 \times 10^{-5}$ M or by substituting Co^{2+} for Ca^{2+} (Fig. 2*d*). The Na^+ current has a transient peak and then inactivates although the time course may vary among different neurones.

After TTX or $Tris^+$ substitution for Na^+, the inward current was carried by Ca^{2+} (Fig. 2*c*). A component of the Ca^{2+} current elicited by small depolarisations showed little or no inactivation as previously reported[19]. At larger voltages the inward Ca^{2+} current also had a transient peak. The calcium was increased or decreased when extracellular calcium was doubled or halved, and reduced or abolished by when Co^{2+} was substituted for Ca^{2+} or verapamil was added. In TTX, the Ca^{2+} current was unchanged when $Tris^+$ was substituted for Na^+. Substitution of methanesulphonate ion for Cl^- had no effect on any of these currents.

Having established the presence of a TTX-sensitive Na^+ current, we tested the effects of trypsin (type III, Sigma) on this current in 20 neurones. The outward K^+ and inward Ca^{2+} currents were blocked and the inward Na^+ currents elicited by 20-mV voltage steps from −60 to +160 mV were recorded. Such Na^+ currents could be blocked by TTX or substitution of $Tris^+$ for Na^+ (Fig. 3*a*). Neurones with the inward Na^+ current separated in this way were then exposed to 0.1% trypsin for about 2–4 min. Exposure to these doses of trypsin for this period increased membrane resistance silghtly but did not affect the magnitude of the inward currents or their reversal potentials. In some instances, the time course was slightly altered. TTX now had no effect on the Na^+ current (Fig. 3*d*). But substitution of $Tris^+$ for Na^+ still blocked the inward current (Fig. 3*e*).

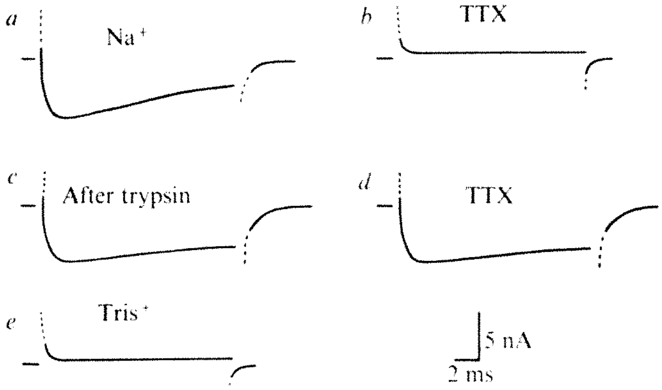

Fig. 3 Na^+ current in a *Helix* neurone, its blockage by TTX and the effects of trypsin on the Na current and the TTX blockage. $V_H = -60$ mV. All currents elicited by voltage steps to +25 mV. Internal perfusion with Cs aspartate, external solution contains CsCl (see text). Ca^{2+} currents blocked by verapamil. *a*, Control Na^+ current. *b*, After 5 min in 3×10^{-5} M TTX. Only the outward leakage current persists. *c*, After recovery in TTX-free solution for 30 min and exposure to 0.1% trypsin for 3 min. The magnitude of the inward current is unchanged but inactivates more slowly. This is not, however, a consistent finding. *d*, After 10 min exposure to 1.5×10^{-4} M TTX in trypsin-free solution. TTX no longer blocks Na^+ current. *e*, Substituion of $Tris^+$ for Na^+ in the external solution eliminates the Na^+ current leaving a small outward leakage current.

To examine whether trypsin acted on the Ca^{2+} current, we separated it from the Na^+ and K^+ currents and compared the inward currents before and after enzyme treatment. Equivalent exposures which abolished TTX-sensitivity had no effect on either calcium currents or their blockage by verapamil or Co^{2+}. Exposures of 10 min, however, reduced membrane resistance and Ca^{2+} currents as well as the TTX-insensitive Na^+ current.

Trypsin destroys the TTX-sensitivity of the neuronal membrane without affecting the magnitude and time course of the inward currents. This accounts for the absence of TTX sensitivity in snail neurones[13] pretreated with this enzyme. The action is specific since Na^+ current persists and the Ca^{2+} current is unaffected. The external application of trypsin to lobster giant axon[1], or α-chymotrypsin to squid giant axon[4], was also without effect on the membrane's electrical properties although another enzyme, Pronase, when applied internally to squid giant axon, removes Na^+ inactivation selectively[3]. If the guanidinium group of the TTX molecule blocks the Na^+ channel at the selectivity filter[20], the persistence of a TTX-insensitive inward Na^+ current with an unchanged reversal potential suggests that the selectivity filter has not been affected by trypsin. Thus, trypsin may act on the part of the channel occupied by the remainder of the TTX molecule and this portion probably contains lysine and/or arginine residues. It is also interesting that modifications of TTX at carbon positions 4 and 9, which are close to the guanidinium group, greatly reduce the potency of the toxin[21]. The lack of TTX sensitivity of some excitable cells, such as puffer fish neurones[22] and denervated skeletal muscle[23] cells, may have a similar basis.

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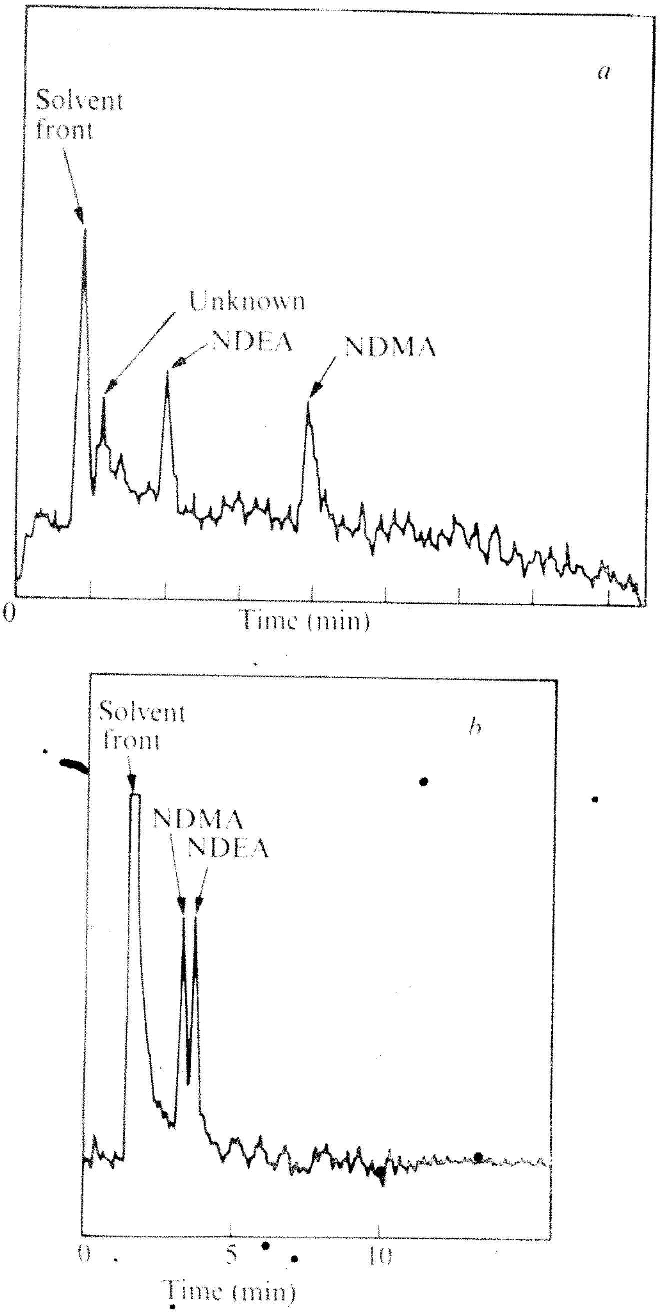
Formation *in vivo* of volatile *N*-nitrosamines in man after ingestion of cooked bacon and spinach

N-NITROSAMINES, which are thought to be causally related to human cancer[1], have been found in μg kg^{-1} concentrations in polluted air[2,3], in water[4] and in tobacco products[5]. Foodstuffs such as cooked bacon, preserved with nitrite, have long been known to contain μg kg^{-1} amounts of volatile *N*-nitrosamines formed during cooking[6]. *In vivo* formation of *N*-nitrosamines, after ingestion of suitable amine precursors and nitrite, has been demonstrated in laboratory animals[7]. *In vivo* formation in human subjects with hypoacidity, gavaged with relatively massive amounts of diphenylamine plus nitrite, has been demonstrated through detection of *N*-nitrosodiphenylamine in the stomach contents[8]. *In vivo* nitrosation after ingestion of conventional foods has not been demonstrated so far, either in animals or man. We now report *in vivo* formation of volatile *N*-nitrosamines in man after ingestion of a midday meal consisting of a bacon, spinach and tomato sandwich and beer.

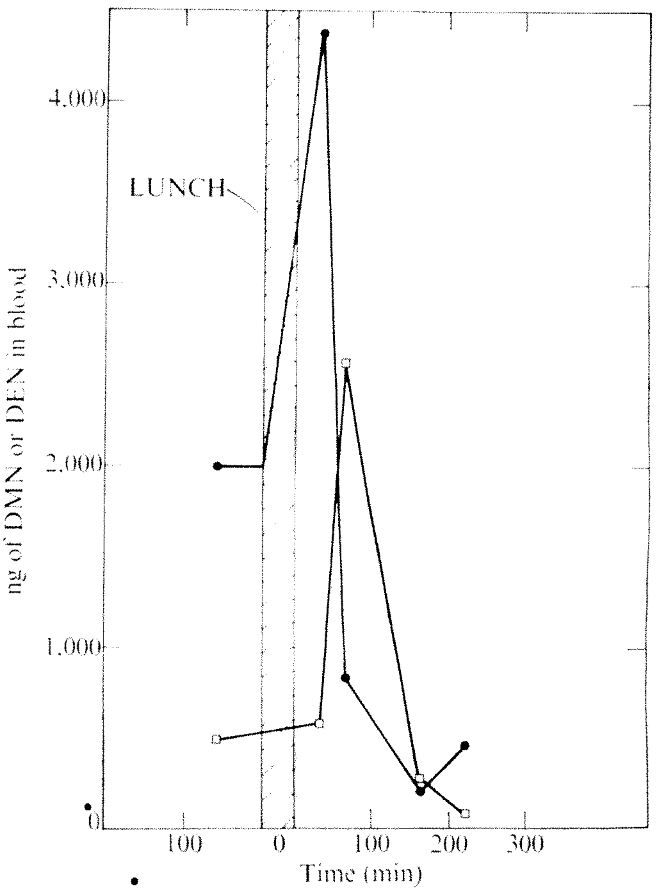
Fig. 1 *a*, High-pressure-liquid-chromatogram for a 10-µl extract of whole human blood. The TEA-HPLC was constructed[10] by combining an injector (Waters Associates, model UK6), with a high-pressure pump (Waters Associates, model 6000A) and a thermal energy analyser (Thermo Electron, model TEA-HPLC 502). A µm Porasil column was used with a 5% acetone–95% 2,3,4-trimethylpentane solvent system at a flow rate of 2 ml min^{-1}. TEA attentuation was × 2. *b*, Gas–liquid chromatogram for a 10-µl extract of whole human blood. A single-column isothermal gas chromatograph was constructed[11] from 14 feet of stainless steel tubing (outer diameter 1/8 inch), packed with 10% Carbowax 20 M on Chromasorb W, 80–100 mesh. The column temperature was 180 C, with Ar carrier gas at a flow of 25 ml min^{-1}. A thermal energy analyser (Thermo Electron, model TEA-502) was used as the detector. TEA attenuation was × 2.



Blood (20 ml) was taken on the day before the test. On the day of the test, 20 ml of blood was taken 50 min before the noon meal. Lunch consisted of 310 g of spinach, 170 g of cooked bacon, 200 g of tomatoes, 120 g of bread and 460 g of beer. One per cent of the total lunch was analysed for volatile *N*-nitrosamines. Blood samples (20 ml) were taken 35, 65, 162 and 220 min after the meal. A final control sample was taken at 1,300 min. Samples were poured immediately into liquid nitrogen. Less than 20 s elapsed between the end of collection and freezing in liquid nitrogen. Mineral oil was added to the whole blood, which was analysed for volatile *N*-nitrosamines by standard procedures[9]. Control experiments were performed by recovering *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA) and *N*-nitrosopyrrolidine (NPYRR) at the 0.4-µg kg^{-1} level (0.4 p.p.b.) from whole human blood (obtained from a blood bank). The blood from the bank contained no voltaile *N*-nitrosamines.

The meal contained preformed NDMA and NPYRR. The total amount of preformed NDMA ingested was 1,600 ng. Identification of NDMA and NDEA was confirmed using both TEA-GLC and TEA-HPLC. Figure 1 shows the chromatographic traces of the blood sample taken 162 min after the meal. The TEA-GLC trace shows only two peaks, those at the retention time of NDMA and NDEA. The TEA-HPLC trace shows three peaks, two eluting at the same retention time as NDMA and NDEA (note that the peak order is reversed). Furthermore, the quantities of NDMA and NDEA are identical for both the GLC and HPLC systems. As a further confirmatory step, the NDMA and NDEA peaks eluting on the HPLC were

Fig. 2 *In vivo* nitrosation in man after ingestion of bacon, spinach, tomato and beer. ●, NDMA; □, NDEA.



isolated and reconcentrated and then injected on to the GLC. The peak corresponding to NDMA on the HPLC gave only a single peak on the GLC, corresponding to NDMA. The same was true for NDEA. Given the selectivity of the TEA, evidence for the identity of volatile *N*-nitrosamines based on parallel TEA-GLC and TEA-HPLC procedures can be taken as confirmatory[10,11].

The amount of NDMA and NDEA present as a function of time in the blood (assuming total body blood is 5,640 ml) is shown in Fig. 2. Before the meal the blood contained 2,000 ng of NDMA and 510 ng of NDEA; 35 min after the meal NDMA had increased to 4,350 ng and NDEA to 570 ng, and 65 min after the meal, NDMA had fallen to 860 ng while NDEA had increased to 2,600 ng. At 162 min, both had decreased below the levels observed before the meal. At 1,300 min there was 760 ng of NDMA in the blood; NDEA was not present. NPYRR was not detected in the blood either before or after the meal.

These amounts of NDMA in human blood imply that the compound was being formed *in vivo* in quantities greater than those present before the meal. The total amount of NDMA in the body may be considerably in excess of that reported here because we assumed that all NDMA was taken up in the blood alone—an appreciable amount may have been exhaled or absorbed in various tissues. NDEA was not found in the food, but it was formed *in vivo* after the meal. It is interesting that the content of NDEA was maximal after the concentration of NDMA had reached its maximum. Our experiments were carried out three times, giving similar results for NDMA, although the actual measurements differed in each case. NDEA was detected in only one of the three experiments. The source of the background NDMA in the blood is unknown; no background was observed if the volunteer had eaten a bland low nitrate–nitrite, high ascorbate diet for the previous 24 h.

The experiments reported here, although simple, demonstrate conclusively that *N*-nitrosamines can be formed *in vivo* in man after ingestion of conventional foodstuffs.

The ability routinely to detect nitrosation *in vivo* should make possible realistic tests of putative environmental carcinogens.

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Ferritinaemia in cancer

SMALL amounts of ferritin are found in serum in normal and pathological states[1,2], usually at levels directly related to the amount of tissue storage iron[3,4]. This relationship, however, does not hold in many cancers where grossly elevated levels can occur without a corresponding increase in iron stores[5–9]. These latter findings suggest a possible use of ferritinaemia in cancer diagnosis, but the value of this test has not been clearly established. Such use is complicated by the multiple potential sources of the serum ferritin which could affect its type as well as its amount. Much of the ferritin in cancer sera may reflect increased iron stores arising from chronic anaemia or from transfusions in treated patients. Some may also come from nonspecific tissue damage. The high levels of ferritin synthesis in some cancer cells[10] and the presence in serum of isoferritins characteristic of some cancer and foetal tissues[11,12], however, suggest that much of the ferritin may come directly from cancer cells. If so, selective quantitation of these isoferritins may provide specificity in possible serodiagnosis. The multiple isoferritins found in most human tissues seem to be hybrid molecules composed of different proportions of two subunit types[13]. One type predominates in liver and spleen isoferritins, and the other in the more acidic isoferritins in heart, tumours and HeLa cells[14]. Different isoferritin populations can be distinguished immunologically, apparently on the basis of their subunit composition[14,15]. Ferritin is usually assayed by immunological methods, with crystalline liver or spleen ferritins as immunising antigens and reference standards. These ferritins consist almost entirely of the liver-type subunit[15]. Consequently, most assays based on these ferritins have only a low reactivity for the more acidic 'carcinofoetal' isoferritins which may contain less than 20% of the liver-type subunit[11,15]. We have, therefore, tested a variety of cancer sera and compared the apparent levels of ferritin given by a standard 'liver-type' assay with levels given by an assay whose specificity is directed against the more acidic isoferritins[15]. We report here preliminary evidence that the latter type of assay may significantly improve the usefulness of ferritinaemia as a tumour marker.

Serum samples from untreated patients with various histologically confirmed malignancies were provided by Dr Elliot Alpert, Massachusetts General Hospital, Boston, Mass. The leukaemic sera were obtained from New England Medical Center, Boston. The samples were not graded according to the stage of disease or other pathological disorders. The levels of ferritin in these sera were estimated by two radioimmunoassays (RIA). One RIA reacts preferentially with liver-type isoferritins, the other with the more acidic isoferritins characteristic of heart and HeLa cells[15]. Antibodies for the 'liver-type' RIA were raised in rabbits against crystalline human liver ferritin consisting of approximately 90% of the liver-type subunit. Antibodies for the 'HeLa-type' RIA were developed against HeLa ferritin (>80% heart-type subunit). Before use, the anti-HeLa antiserum was absorbed with a natural apoferritin preparation (>95% liver-type subunit) to restrict its specificity to sites determined by the heart-type subunit[15].

The apparent level of the serum ferritin from 8 out of 12 different cancers was substantially higher when estimated with the 'HeLa-type' than with the 'liver-type' assay (Table 1). In most cases, the ratio of the values given by the two assays (HeLa–liver) was between 2 and 5. These ratios are markedly different from those obtained in non-malignant conditions, such as iron overload, where the ratio was less than 0.1. This differential indicates that the ferritin in these cancer sera contained substantially higher proportions of the heart-type subunit than the serum ferritins from iron overload. This conclusion must also apply even in cases where the apparent level given by the 'HeLa-type' RIA was slightly lower than that given by the 'liver-type' RIA (for example, cases 4, 21).

Table 1 Serum ferritin levels (ng ml^{-1})

Case no.	Diagnosis	Liver-type	HeLa-type	Ratio HeLa/liver
1	Normal	77±48	ND	—
2	Hemochromatosis	5000	190	0.04
3	Hemochromatosis	950	ND	—
4	Teratoblastoma	1550	1000	0.6
5	Glioma	130	ND	—
6	Ovarian carcinoma	62	430	7.1
7	Breast cancer	205	500	2.4
8	Breast cancer	330	800	2.4
9	Breast cancer	190	350	1.8
10	Breast cancer	50	140	2.8
11	Pancreatic carcinoma	600	1900	3.1
12	Pancreatic carcinoma	320	600	1.9
13	Pancreatic carcinoma	460	500	1.1
14	Hepatoma	560	850	1.5
15	Gall bladder carcinoma	550	2700	5.0
16	Colon carconoma	220	1500	6.7
17	Stomach carcinoma	750	1750	2.4
18	Stomach carcinoma	118	235	2.0
19	Stomach carcinoma	550	1050	1.9
20	Lung carcinoma	630	1750	2.8
21	Multiple myeloma	2630	1350	0.5
22	Acute lymphoblastic leukaemia	3800	165	0.04
23	Acute lymphoblastic leukaemia	34670	130	0.004
24	Acute myeloblastic leukaemia	1675	ND	—
25	Acute myeloblastic leukaemia	1866	135	0.07
26	Pancreatitis	1170	740	0.6

Values given as ND were below the minimum detectable level at 100 ng ml^{-1} in the 'HeLa-type' assay. The difference in sensitivities of the two assays reflects our inability to iodinate HeLa ferritin to the same extent as liver ferritin.

Although the differential in serum ferritin levels given by the two assays holds in most cancers examined here, some, such as the leukaemias, did not conform to this general pattern. The very low HeLa–liver ratio in these samples could reflect liver-type isoferritins in the cancer cells or increased iron stores due to deficient erythropoiesis. High levels of the more acidic isoferritins have been found in untreated leukaemias[8].

The immunological differences among human isoferritins might be of value in obtaining information about serum ferritin phenotypes in various pathological states. Our results indicate that the 'HeLa-type' RIA might not only add a key element of specificity for assessing ferritinaemia in many cancer sera but might also detect abnormally high levels which might not be evident with the 'liver-type' assay (for example, cases 6, 7, 9, 16). If the more acidic isoferritins reflect high levels of secretion, rather than cell damage, their detection may prove useful in early diagnosis. It should be noted, however, that elevated levels of these isoferritins in sera are unlikely to be entirely specific for cancer. These isoferritins occur in small amounts in heart, kidney and pancreas[16] and their level in serum may increase in conditions such as myocardial infarction[5,8] and pancreatitis (case 26). More thorough studies are required to extend our preliminary observations and to correlate the type and amount of serum ferritin in cancer and other pathological states throughout the course of the disease.

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Phosphorus nuclear magnetic resonance of perfused working rat hearts

THERE are serious limitations to techniques used to correlate biochemical parameters with function in metabolically active tissues. For the perfused heart such techniques include measurement of surface NADH fluorescence[2], comparison of coronary arterial–venous composition, and biochemical analysis of intracellular metabolites from biopsy specimens. Although the latter approach has yielded critical information, it is limited because local injury prevents accurate interpretation of mechanical function. Recently ^{31}P nuclear magnetic resonance (NMR) has been used to analyse excised muscles and their acid extracts for intracellular concentrations of the sugar phosphates, glycolytic intermediates, free orthophosphates, phosphocreatine, ADP and ATP. In addition, the *p*H-dependent chemical shift of the orthophosphate resonance has been titrated[2] and used to determine the intracellular *p*H of intact muscles[3]. These muscles were neither perfused nor functioning, but we report here the assessment by ^{31}P NMR of the myocardial energy status in the working, perfused rat heart subjected to various degrees of ischaemia.

Albino Sprague–Dawley rats (200 g) were injected with 1,000 U of heparin and anaesthetised with 20 mg of pentobarbital. The hearts were excised, washed with cold perfusate and, within 3 min, perfused by means of a cannula inserted into the aorta and positioned above the aortic valve. The cannula was attached to a column 107 cm high. Left ventricular pressure was monitored by means of a catheter, 100 cm long, inserted through the left atrial appendage and mitral valve, and connected to a Statham P23Db strain gauge tranducer and a Brush 220 recorder. The perfusate was maintained at 30 °C and was bubbled continuously with pure oxygen. It contained 145 mM NaCl, 6 mM KCl, 1.3 mM $CaCl_2$, 0.4 mM $MgSO_4$, 16.7 mM glucose, 20 mM Tris-Cl (*p*H 7.4) and 1% Dextran 70.

^{31}P NMR spectra were obtained on a Nicolet TT 23 pulsed Fourier transform spectrometer interfaced to a Varian V 4412 probe. The heart was contained in a 12-mm NMR tube. Field-frequency control was obtained by locking on the deuterium signal from a 3-mm glass tube, containing pure D_2O, which was slipped into the NMR tube alongside the pulsing heart. Where desired, an external reference signal was obtained from a 1-mm capillary containing 0.2 M H_3PO_4 in 15% $HClO_4$. Typically, data was obtained using an 8K data table, a 2,000-Hz spectral width, a 2.0-s acquisition time and 1,000 transients. Probe temperature was maintained at 30 °C.

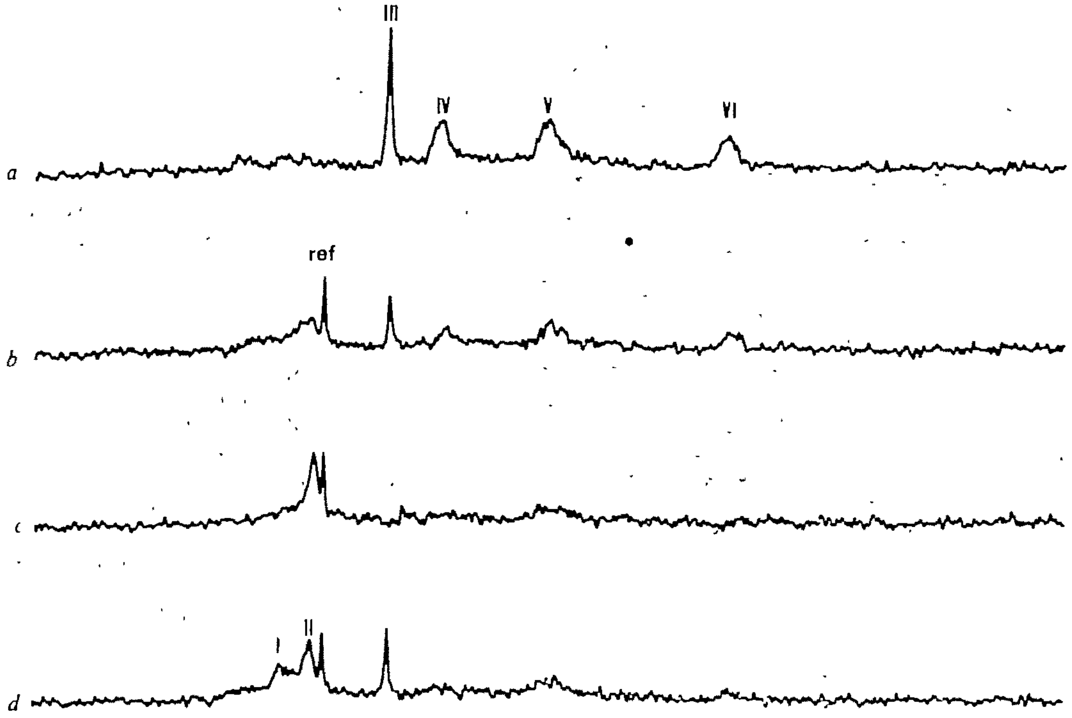

Fig. 1 ^{31}P NMR Spectra of working perfused rat hearts (40.5 MHz). *a*, Normal perfusion pressure of 107 cm H_2O. *b*, partial ischaemia, at a perfusion pressure of 43 cm H_2O Ref., Reference signal. *c*, Total ischaemia with aortic cross-clamp. *d*, Reperfusion at 107 cm H_2O. Peak assignments: I, sugar phosphates; II, orthophosphate; III, phosphocreatine; IV, γ ATP; V, αATP, VI, βATP,

Figure 1*a* shows the ^{31}P spectrum of a beating perfused rat heart in optimum conditions of perfusion. Assignments are taken from ealier work on excised muscle[2,3]. The active muscle is characterised by prominent resonances from the three phosphates of ATP and the phosphate group of phosphocreatine and only weak resonances from inorganic phosphate and sugar phosphates. When partial ischaemia was produced by reducing the perfusion column from 107 to 42 cm H_2O, the heart rate declined from 214 to 178 beats per min and left ventricular pressure declined as expected (Fig. 1*b*). The intensities of the ATP and phosphocreatine resonances decreased markedly relative to that of inorganic phosphate. Figure 1*c* shows the effect of 25 min of total ischaemia produced by cross clamping the aortic cannula. During total ischaemia the heart stopped pulsing. Neither phosphocreatine nor ATP resonances were detectable but the inorganic phosphate resonance increased markedly. With reperfusion the heart started to beat again, and the resulting ^{31}P spectrum is shown in Fig. 3*d*. Obviously, the phosphocreatine and ATP resonances increased above the ischaemic levels while the inorganic phosphate resonance decreased. Apparently, the total intensity of the phosphorus resonance decreased with time, presumably due to leakage of inorganic phosphate from the heart into the perfusate during ischaemia. The *p*H of the tissue was monitored by the chemical shift of inorganic phosphate. The results for the heart in various conditions are shown in Table 1. Intracellular *p*H decreased by 1.2 *p*H units during partial ischaemia and by a further 0.5 *p*H unit during total ischaemia.

In several experiments (spectra not shown) the linewidth of the P_i resonance tended to be greater in fully perfused heart than in either partially or totally ischaemic heart. This suggests that *p*H gradients existing in oxygenated heart are dissipated in ischaemia. During reperfusion of ischaemic heart a small signal appeared in the region expected for sugar phosphates. This may reflect a buildup of glycolytic intermediates before some critically inhibited enzymatic step.

Table 1 Intracellular *p*H measured by ^{31}P NMR

Condition	Perfusion pressure (cm H_2O)	Chemical shift (p.p.m. from H_3PO_4–$HClO_4$ reference)	*p*H
Normal	107	2.04	7.4
Partial Ischaemia	43	0.86	6.2
Total Ischaemia	0	0.46	5.7
Reperfusion	107	0.68	6.1

Thus we have explored both the energy status of a tissue and its intracellular *p*H. Biochemical data were collected in steady-state conditions and correlated well with changes in mechanical function. With minor modifications our method is applicable to liver, kidney and other perfused tissues, and we believe that it represents a useful new technique.

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Cloning and expression of the flagellar hook gene on hybrid plasmids in minicells

SPECIFIC *Escherichia coli* DNA fragments carrying flagellar genes have been cloned on λ vehicles and gene expression has been studied in λ-infected ultraviolet irradiated-cells[1-4]. This work has led to the identification of several gene products involved in flagellar function. But, the structural gene for the hook subunit protein, which is one of the major flagellar components has not been identified. This gene could have been missed if it was not part of the DNA that was cloned, if it was cleaved during endonuclease treatment or if it was transcribed at such a low level that the product could not be detected in ultraviolet-irradiated cells. We report here another approach which avoids these difficulties and has enabled us to identify the region of the genome which carries genes involved in the synthesis of flagellar hook and basal structures. This result is interesting for two reasons. First, it demonstrates the general applicability of cloning techniques to the identification of any gene product in *E. coli* Second, it focuses attention on a hitherto poorly defined group of genes involved in flagellar structure and function.

Various proteins are associated with *E. coli* flagellar structure and function. The flagellar organelle seems to be composed of three sections: the filament, the hook and the basal structure[5-7]. The filament is made up of a single polypeptide subunit called flagellin with an apparent molecular weight of 53,000 (ref. 6). The hook is also composed of a single polypeptide subunit with a molecular weight of 42,000. The hook subunit is clearly distinguished from flagellin by its specific reaction with antihook subunit antiserum[7]. The flagellar filament rotates, and the hook may act as a flexible 'universal joint' connecting the filament to the basal structure. The basal structure is complex and composed of at least nine different polypeptides[8]. Other proteins involved in flagellar formation and function are localised in the cell membrane and cytoplasm[2,9].

The genes responsible for flagellar formation have been defined by genetic complementation tests, and more than 20 of them were found to map in three regions of the *E. coli* chromosome (Fig. 1). Some of these genes have regulatory functions. Thus strains that carried mutations in the *flaI* gene did not synthesis hook subunits or other flagellar component proteins[7,9]; whereas strains that carried mutations in the *flaE* gene seemed to over-produce the hook subunit protein and formed polyhooks. The *flaE* gene is not responsible for the synthesis of the hook subunit protein[7,10]. Mutants that have altered hook subunit proteins have not been found. To find the structural gene, it was necessary to use the plasmid–*E. coli* hybrid system and study the expression of genes carried by plasmids that were segregated into minicells.

Clark and Carbon[11,12] prepared a colony bank of strains

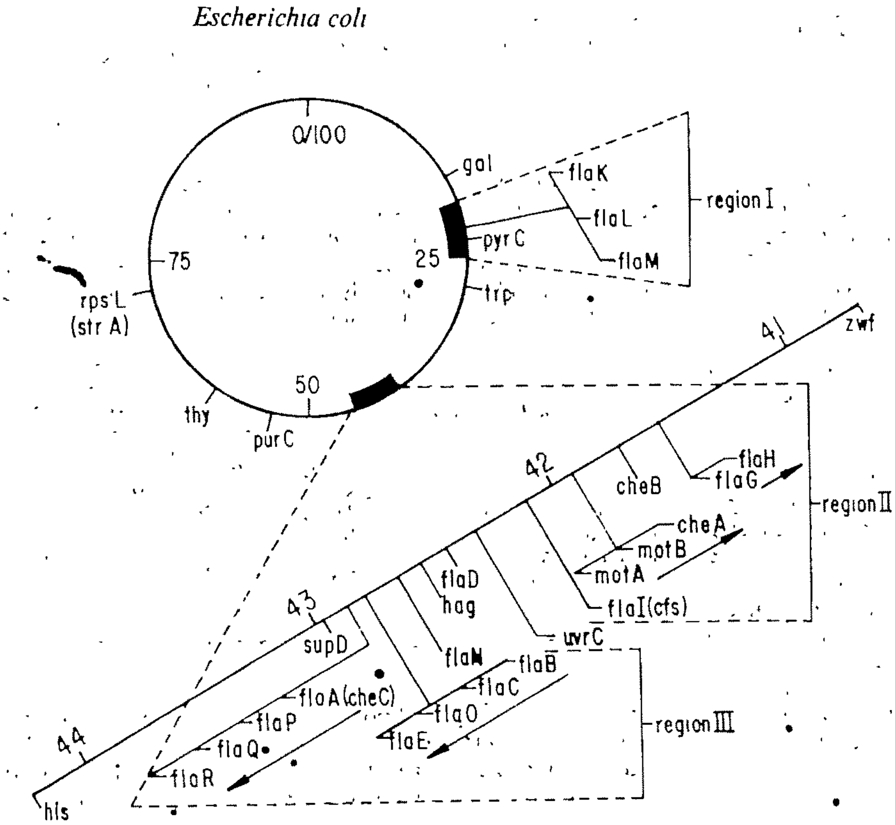


Fig. 1 The map positions of genes controlling flagellar structure and function in *E. coli*. The gene designations have been described before[8,14]. fla refers to genes that include mutation which result in strains that have no apparent flagellar structure; hag is the structural gene for the major subunit protein of the flagellar filament; mot refers to mutations that result in paralysed flagella; che includes strains that do not respond to chemotactic signals; cfs refers to a regulatory locus controlling the sensitivity of flagellar formation to cyclic AMP (ref. 7).

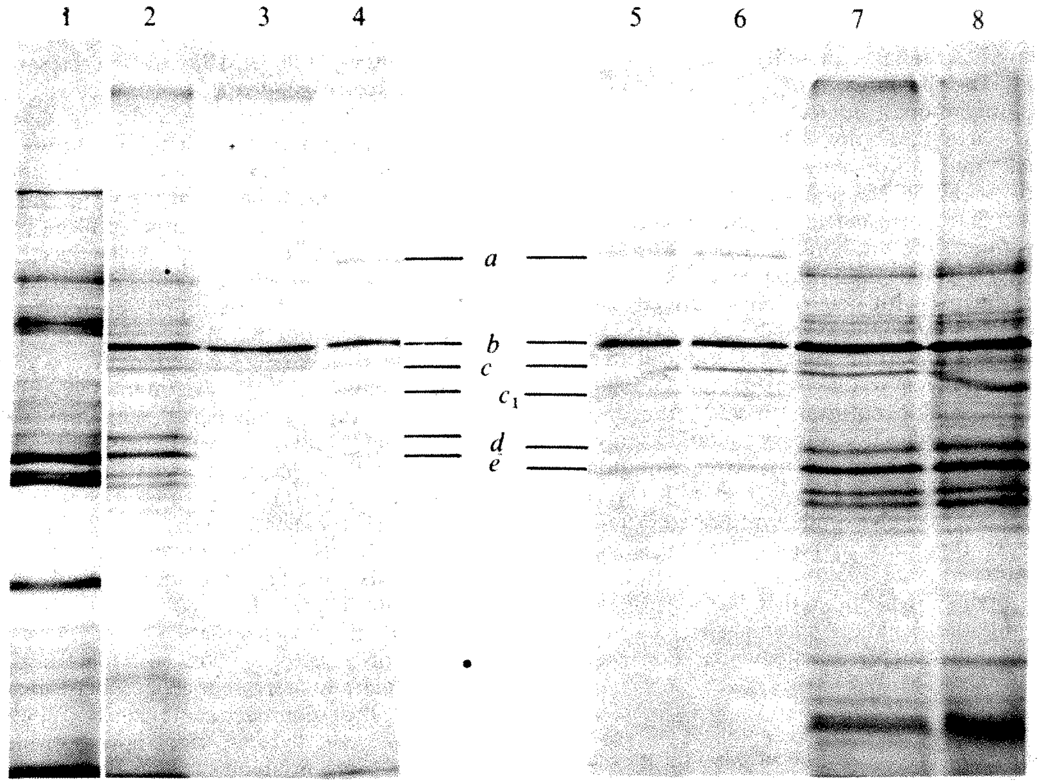


Fig. 2 Autoradiograms of SDS–acrylamide electrophoresis gels of minicells carrying hybrid plasmids. The plasmids were transferred by conjugation into minicell producing strain P678-54 (obtained from R. Sparks, University of California, San Diego). All plasmid-carrying strains were from the Clarke and Carbon collection[12]. Minicells were collected after the parent strain had been grown for 14 h in L broth and thymine (15 μg ml^{-1}) at 37 °C. The minicells were purified by differential centrifugation and sucrose gradients[13]. They were pre-incubated in M-9 minimal medium with glycerol supplemented with 19 amino acids (4 μg ml^{-1}); no methionine was added. After incubation at 37 °C for 20 min, ^{35}S-methionine (20 μCi ml^{-1}, 350 Ci mmol^{-1}) was added and incubation continued for 30 min. The minicells were collected and lysed in buffer containing 3% SDS and 0.5 M βME. Samples were heated to 100 °C for 2 min. The electrophoresis on 12 1/2% acrylamide gels has been described before[2]. In samples 5 and 8, βME was omitted. For antibody precipitation, the minicells were lysed by treatment with EDTA and lysozyme[8] and then sonicated for 2 min. The debris was removed by centrifugation for 45 min. The supernatant was dialysed, lyophilised and reconstituted, and nonradioactive hook subunit protein was added as carrier. Anti-hook subunit antibody 926 (ref. 7) was used to precipitate the protein at equivalence. The precipitate was washed twice with cold buffered saline and resuspended in lysis buffer. Sample 1, minicells with plasmid pLC 7-18 carrying *flaD*; sample 2, minicells with plasmid pLC 11-15 carrying *flaK*, *flaL* and *flaM*; sample 3, pellet after antibody precipitation of extract of minicells carrying pLC 11-15; samples 4 and 6, hook and basal structure components prepared as previously described[6]; samples 5, same as 4 and 6, except that no βME was added; sample 8, same as 2 and 7, except that no βMe was added. The letters in the centre refer to the position of some of the components of the basal structure that are found after the purified hook–basal structure[8] is dissociated. *a*, Component with apparent molecular weight of 60,000; *b*, hook subunit protein, *c* and c_1, positions assumed by component in the presence and absence of added βME; *d*, component with apparent molecular weight of 30,000; *e*, 27,000 molecular weight component.

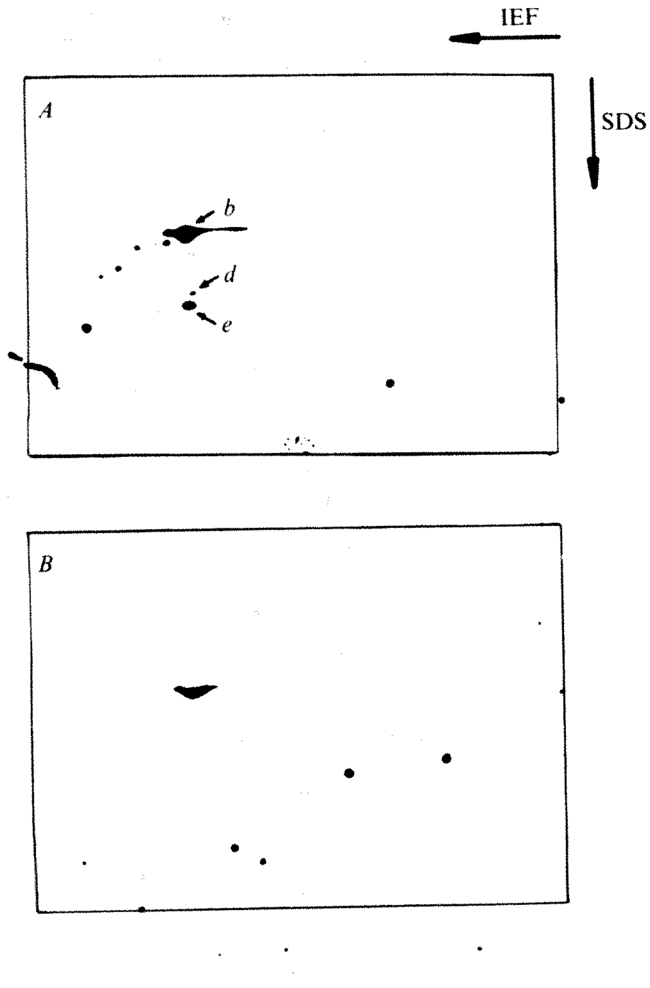
with hybrid *E. coli*–colicinogenic factor E_1 (Col E1) plasmids. Each hybrid carried a randomly selected fragment of *E. coli* DNA so that the bank, consisting of more than 2,000 clones, carried within it all the *E. coli* genes. Furthermore, each strain had an F factor which allowed the transfer of the hybrid plasmid. These strains were tested by mating into various *fla*⁻ recipients on motility plates. The clones that were able to transfer motility were further characterised by genetic complementation. Sixteen strains were found that carried plasmids with one or more of the known flagellar genes[12]. There were four in region I, seven in region II and five in region III. These strains were used to transfer the plasmids into a minicell-producing strain. Minicells were prepared[13] and purified by multiple sucrose gradients. The minicells were then incubated with ^{35}S-labelled methionine, and after incubation, the total minicell pellet was disassociated, and the peptides were separated by acrylamide gel electrophoresis. Different minicell preparations had different levels of contamination with whole cells. Contamination resulted in a low level of incorporation of label into a large variety of polypeptides. These formed very similar patterns of distribution on the gel, independent of the nature of the plasmid carried by the parent strain (Fig. 2, columns 1 and 2). On the other hand, there were specific, heavily labelled polypeptide bands that were characteristic of the hybrid plasmid carried by the minicells.

Thus far, minicells from strains that carried a plasmid with *fla* genes mapping in region III (pLC 24-16) were found to synthesise a polypeptide with the same mobility on acrylamide gels as flagellin. Plasmids that carried the *cheB* genes in region II (pLC 21-2 and pLC 24-15) directed the synthesis of polypeptides with the same electrophoretic mobility as the *cheB* gene products synthesised by hybrid *E. coli*–λ bacteriophage (our work, in preparation). Another plasmid carrying the *flaD* gene (pLC 7-18) led to the synthesis of distinctive polypeptides (Fig. 2, column 1) that have not yet been assigned to specific genes. Among the plasmids that have been tested, only those that carried genes in region I (pLC 11-15, pLC 36-11, pLC 24-46 and pLC 35-44) led to the synthesis of a band with the same electrophoretic mobility as the hook subunit (Fig. 2, column 2 and 4). In fact, four of the labelled bands *b*, *c*, *d* and *e* have the same mobilities as polypeptides derived from the purified hook–basal structure complex[8,14]. These bands correspond to molecular weights of 42,000, 38,000, 30,000 and 27,000.

To determine whether the band with a mobility corresponding to a molecular weight of 42,000 was the same as the hook subunit protein, a minicell extract was prepared and treated with anti-hook subunit antiserum. The precipitate was washed and separated by acrylamide gel electrophoresis. The major radioactive polypeptide found in the precipitate has the same mobility as the hook subunit (Fig. 2, column 3). The same experiment was performed using the two-dimensional isoelectric focusing technique described by O'Farrell[15]. Figure 3*A* shows that there are several different radioactive polypeptides. Spot *b* corresponds precisely with the position of nonradioactive hook subunit protein that was added to the mixture. After precipitation with specific antihook antibody, this spot was the only radioactive polypeptide found on the gel (Fig. 3*B*). In control experiments with heterologous antibody or with the specific antihook subunit antibody and extracts containing labelled proteins from different minicell preparations, no specific precipitation was found. Finally, in experiments using double diffusion in agar, the antihook subunit formed a specific line of precipitation only with the radioactively labelled material in extracts of minicells carrying the plasmids with genes in region I. Thus, the 42,000 molecular weight polypeptide synthesised in minicells carrying *fla* genes that map in region I behaves like the hook subunit protein.

Some of the other polypeptides that were synthesised in minicells carrying the region I plasmids were similar to other flagellar basal structure components. For example, the 38,000-molecular weight basal structure component had the distinctive property that its mobility on sodium dodecyl sulphate (SDS) gels was affected by the presence of β-mercaptoethanol (βME). In the presence of βME, its mobility corresponded to a molecular weight of 38,000 while in the absence of reducing agent, its mobility corresponded to a molecular weight of 36,000 (Fig. 2, column 6 and 5, bands *c* and c_1). The polypeptide synthesised in minicells behaved in the same way (Fig. 2, column 7 and 8, bands *c* and c_1). Furthermore, the 38,000 molecular weight component of flagellar basal structures is not found on the two-dimensional gels[6]. It is presumably lost during isoelectric focusing. The corresponding band in the minicell preparation also does not appear on two-dimensional gels (Fig. 3*A*). Finally, polypeptides *d* and *e* (Fig. 2 and Fig. 3*A*) have mobilities very similar to the basal structure components with molecular weights of 30,000 and 27,000 on two-dimensional gels[6].

Fig. 3 Autoradiograms of two-dimensional gels. Minicell extract for antibody precipitation was prepared as described in legend to Fig. 2 from minicells carrying pLC 11-15. The gel technique was that described by O'Farrell[16]. The left side of the gel is the acidic end of the isoelectric focusing run and the right side the basic end. *A*, whole extract before antibody precipitation; *B*, after antibody precipitation.



We conclude that a gene that maps in region I specifies the structure of the hook subunit protein. The polypeptide product of the gene carried on the hybrid plasmid was identified by its motility on one- and two-dimensional gels and by its specific precipitation with antihook subunit antiserum. Three other polypeptides with characteristics similar to the basal structure components with molecular weights of 38,000, 30,000 and 27,000 are also synthesised by the plasmid carrying genes in region I. This finding extends the approach previously described involving lambda-*E. coli* hybrids. It is now possible to identify all flagellar gene products by programming their synthesis with cloned fragments of *E. coli* DNA. Initial genetic studies on region I have identified three (*flaK, flaL, flaM*) flagellar specific complementation groups[16]. Further studies are required to define completely the genes in this region and to understand the regulatory mechanisms that control the expression of these genes and coordinate it with the other flagellar genes.

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matters arising

Rock volatility and aerosol composition

GOLDBERG[1] has suggested the possible importance of contributions to the atmospheric burden of trace components from the sublimation of surface minerals. We show here, however, that the observed trace element concentrations could only be achieved by higher temperatures than those encountered on the Earth's surface. Goldberg used a simple kinetic model, where the rate of loss of substance per unit area φ from a pure surface under a vacuum is equivalent to the rate at which vapour molecules would reach the surface when the solid is in equilibrium with its vapour[2] and may be written

$$\varphi = p_0 \, (M/2\pi RT)^{\frac{1}{2}}$$

where p_0 is the equilibrium vapour pressure, M the molecular weight and R, T have their usual meanings. This represents the maximum rate at which molecules can leave the surface. In the presence of other gases, however, the rate will be lower owing to the existence of a boundary layer and transfer of vapour molecules across this layer will be the rate determining step in most cases. The rate of diffusion across a laminar boundary layer from 'Fick's Law' is

$$\varphi = \frac{DM}{RT} \frac{\partial p}{\partial x}$$

where D is the diffusion coefficient and x is distance. Trace component concentration in the atmosphere will be low as they are removed rapidly from the bulk atmosphere by convective and chemical processes, $(\partial p / \partial x)$ can be approximated by $p_0 / \Delta x$, where Δx is the thickness of the boundary layer (~ 2 mm) (ref. 3). The importance of the diffusion layer can be adequately demonstrated by the example of DDT ($M = 0.354$ kg) where $D = 5 \times 10^{-6}$ m^{2}s^{-1} and $p_0 = 2 \times 10^{-5}$ Pa (ref. 3). The value φ arising from a pure DDT surface is calculated as 7.3×10^{-12} kg m^{-2}s^{-1} which is in good agreement with the experimental rates (ref. 3) of 0.8–5.5 $\times 10^{-12}$ kg m^{-2} s^{-1}, but calculations using the simple kinetic model which neglects the boundary layer suggest rates some four orders of magnitude too high (9.6×10^{-8} kg m^{-2} s^{-1}).

When considering components of a mixed solid such as a mineral, the activity (f) of the component must be considered. Little is known about the activity coefficients applicable to the sublimation of trace components of minerals, but they can be of several orders of magnitude (for example, 10^{-3} to 10^3, ref. 4). In our model unit activity coefficients were assumed and activity was equated with mole fraction. A solid phase resistance to sublimation must also be considered. When a trace element evaporates from the exposed surface of a matrix, the surface region becomes depleted in the evaporating component. Thus the overall rate of loss may well be controlled by the replenishment of the surface region through transfer of the trace component to the surface from the bulk of the solid. Thus there may be two resistances to transfer, one in the solid phase and one in the gas phase, which limit the evaporative flux. Furthermore, solid phase resistance can also occur in pure solids where structural irregularities which control the sublimation must be regenerated[5]. For the minerals of interest here, the magnitude of the solid phase resistance is not easily determined and has been omitted. This yields upper limits to the sublimation rate as far as solid phase resistance is concerned.

When sublimation is the sole contribution to the atmospheric burden, the principle of steady state enables φ to be equated with the deposition rate per unit area CH/τ, where C is the atmospheric concentration, H is the height of the tropopause (about 13 km) and τ is the tropospheric residence time (about 10 d)[1]. Thus the pressure (p^*) needed to account for the observed atmospheric burdens can be calculated from

$$p^* = \frac{CHRT\Delta x}{DMf\tau} \quad (1 \text{ atm} = 10^5 \text{ Pa})$$

Table 1 Properties of some of the elements which behave anomalously in the atmosphere

Element	Atmospheric Concentration (from ref. 6) C (10^{-13} kg m^{-3})	Weight fraction of the crust (p.p.m.) (from ref. 8)	Mole fraction of crust ($\times 10^6$)†	p^* (10^{-6} atm)	T(°C)‡
SeO_2	2	0.05	0.03	4.7	77
As_2O_3	0.5	1.8	0.6	0.03	74
Sb_2O_3	0.3	0.2	0.04	0.18	198
PbO	35	12.5	3.6	0.32	600
CdO	2	0.2	0.1	1.0	680
Hg(1)	5	0.08	0.025	6.6	39

†Using average crustal compositions from ref. 8; the 'average' molecular weight of the crust was computed as 63.5 g mol^{-1}. Weight fractions were then corrected for relative molecular weight.

‡From p_0–T formulae, in ref. 9.

Table 1 shows the results of calculations using this formula for metals that show anomalously high atmospheric concentrations that cannot be accounted for by crustal or marine inputs. For most of the elements discussed here, vapour pressure data for actual crustal minerals is not available, so that measurements for pure solids as indicated are used for provisional estimates. The final column gives the minimum temperature at which the pressure p^* is achieved. For all the oxides, the calculated temperature is outside the range of validity of the p_0–T data. The direct extrapolation, however, overestimates the vapour pressure at a given temperature. It is noticeable that even for the volatile elements (As, Se, Sb) the oxides have insufficient volatility to account for their atmospheric levels at ambient temperatures, although temperatures of nearly 70 °C might be achieved at the surface of minerals in tropical regions. For CdO and PbO the temperatures required are well above ambient, but such temperatures may be achieved in a number of high temperature processes, such as ore roasting or coal burning and in volcanoes. The transfer of the more volatile elements to fly ashes has been noted[7]. As a special case the p^* and T have been calculated for mercury metal, with its high vapour pressure. As can be seen, a temperature of at least 39 °C is needed to account for the observed atmospheric levels if all the crustal burden of mercury existed as the liquid metal. Sublimation of natural mercury ores such as cinnabar (HgS) would, of course be very much slower. Further scrutiny of the p_0–T data indicates that activity coefficients of at least 10^4 and negligible solid phase resistance are needed to account for the anomolously high atmospheric concentrations of As, Se, Sb, Hg, Pb and

Cd by a sublimation mechanism at ambient temperatures. This is not to imply, however, that such processes could not be important at higher temperatures or lower atmospheric pressures, possibly contributing to the atmospheric chemistry of other planets.

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Control of flight activity in mosquitoes

JONES, in his interpretation of the activity cycle of the mosquito *Culex pipiens fatigans* Wied. in constant light (LL) following a light : dark (LD) regime of 12 h light and 12 h dark[1], stated that it was possible to calculate that the first peak should have occurred about 18 h after light-on or 6 h after the normal time of light-off. In his recordings of activity the main peaks in LD 12:12 were immediately following light-off and light-on. In most field observations of biting activity[2–4], in the laboratory cycle of mating activity[5] and in my laboratory observations of flight activity, the main peaks in the daylength (LD 12:12) typical of the tropics are around midnight and after light-on. By plotting the positions of the peaks in various LD regimes I found the control of flight activity in *C.p. fatigans* to be a combination of light-on some 13–18 h earlier and light-off some 8–14 h earlier (Fig. 1). The mosquito will also respond immediately to light-on in LD 8:16 to LD 20:4. I did not study the activity in LL but in constant dark (DD) following an LD 12:12 I observed a bimodal cycle with one peak following light-on by about 18 h and the other following light-off by about 12 h; these were both repeated twice in DD with a period of about 27 h. Clear double peaks were shown in DD following an LD 4:20 rearing regime; the period in this case was about 24 h. The second peak became much greater with successive cycles to present a result resembling the activity in LL as recorded by Jones[1].

The separate phase-setting effects of light-on (dawn) and light-off (dusk) in a mosquito were first observed for *Aedes aegypti* L. and formed the basis for suggesting an association between the range of photoperiods in which these separate phase-setting effects reinforce each other and the photoperiod usually encountered by a species throughout its natural range[6]. For dark-active species, such as *Anopheles farauti* Laveran, the optimum photoperiod seems to be determined by the coincidence of a peak entrained by the previous light-off and the peak in response to light-on[7]. *C.p. fatigans*, to judge from the increase in the size of the light-off entrained peak in DD and LL, probably has the same mechanism for determining photoperiod. A theoretical optimum daylength of 12–14 h for *C.p. fatigans*, derived from Fig. 1. compares reasonably well with its known geographical range of 0°–35/40° latitude.

The relevance of the association between photoperiod measurement and the photoperiods within their natural geographical range is not obvious for tropical mosquitoes but, increasingly in recent years separate 'dawn' and 'dusk' oscillations have been linked with the measurement of photoperiod by insects which have a diapause stage in the annual life-cycle[8]. It has not been clearly established, however, whether overt rhythms of activity can be used as indicator rhythms in photoperiodism studies. I have studied several temperate species of mosquito which have at least two generations per year. For *C. pipiens pipiens* L. I obtained graphs of activity peaks which for adults reared in July from larvae collected in southern England (latitude 51 °N, daylength 15 h), that is, a summer generation, gave a theoretical optimum daylength range of 14–17 h, but for adults taken from hibernation in January, that is, the winter generation, the range was 11–12 h. *C. torrentium* Martini showed a range of 15–18 h for adults reared from larvae collected in mid-July whereas the range for adults reared from larvae collected in mid-September (daylength 13 h) was 12–14 h. The effect of shortening daylengths on summer generation mosquitoes and of lengthening daylengths on winter generation mosquitoes can clearly produce an imbalance between the internal mechanisms controlling flight activity, so there may be a link between overt flight activity rhythms and the photoperiodic mechanism controlling the adult diapause of hibernating mosquitoes.

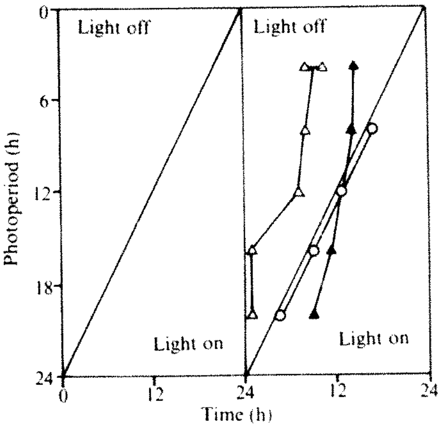

Fig. 1 The positions of the peaks of activity of *C.p. fatigans* relative to light-on and light-off when the mosquitoes are in different light regimes. Diagonal lines divide light-on and light-off periods. Peaks of activity are indicated as: ○, peak at light-on; △, peak following light-on by 13–18 h; ▲, peak following light-off by 8–14 h. Total of 26 individual females was studied; activity was recorded acoustically[6] at 25 °C; light intensity was 70 lx.

This work is part of a Ph.D thesis, Brunel University, 1969. I thank the Ministry of Overseas Development and the MRC for financial assistance and Professor J. D. Gillett for his interest and encouragement.

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JONES REPLIES—Biting and mating depend on flight, but do not necessarily have to show the same pattern; all that is required is sufficient flight activity at the appropriate time. The cycle I have observed in *C.p. fatigans* provides such a permissive framework; it is also consistent with the pattern of flight activity measured in the field using window traps.

Taylor's results[1,2] could indicate an interesting difference in behaviour between strains (his material originated in Lagos). Confirmation of his results would be useful as the experiments he has quoted were all carried out at the same time using only one batch of insects which had been reared together.

'Dawn' and 'dusk' oscillators may indeed play an important part in the control of mosquito flight activity. This would fit in with the new model for circadian pacemakers proposed by Pittendrigh and Daan[3] which they describe as a 'clock for all seasons'.

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[1] Taylor, B. *Nature* **265**, 762 (1977).
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Dirac's continuous creation cosmology and the temperature of the Earth

ROXBURGH[1] has contended that the present form of Dirac cosmology[2] predicts too low a temperature for the Earth (in the recent past) to be acceptable. He computed the time dependence of the temperature of the Earth with the relation

$$T = \left(\frac{L}{R^2}\right)^{1/4} \qquad (1)$$

The solar luminosity $L \sim G^7 \times M_\odot^5 \sim t^3$, when the Dirac prescriptions $G \sim t^{-1}$, $M_\odot \sim t^2$ are used. For the time dependence of the radius of the Earth R in atomic units, Roxburgh proposed and solved the equations of motion ((4) and (4*a*) in ref. 1). These equations are not valid in atomic units. Being the standard Newtonian equations, they are valid only in Einstein units where, since m is constant, the differentiation between (4) and (4*a*) in ref. 1 is meaningless. From Roxburgh's equations, we conclude that the orbital radius in Einstein units $\bar{R}$ = constant.

Dirac[1] has argued that with multiplicative creation, $R \sim \bar{R}t$. Equation (1) then yields $T \sim t^{1/4}$, as shown by Canuto and Lodenquai[3]. Such a time variation cannot be ruled out. While Roxburgh considers Dirac's argument unconvincing, we point out that it is substantiated by dynamic equations of a consistent theory (refs. 4 and 5). In the following, we indicate four equivalent methods by which the correct equation of motion in atomic units can be derived.

(1), With a general transformation of units of the form $d\bar{s} = \beta ds$, where $d\bar{s}$ and ds are the space-time intervals in Einstein and atomic units respectively, the geodesic equation of general relativity transforms into

$$\frac{d}{ds}(\beta v^\mu) + \beta\Gamma_{\nu\lambda}{}^{\mu}v^\nu v^\lambda = g^{\mu\nu}\beta_{\nu} \qquad (2)$$

(2) The gravitational field equations in atomic units given in refs 4 and 5 can be used to derive

$$T_{\mu,\nu}{}^{\nu} = -3\frac{\beta_{,\nu}}{\beta}\left(T_\mu{}^\nu - \frac{1}{3}T_\lambda{}^\lambda\delta_\mu{}^\nu\right) \qquad (3)$$

Using the stress-energy tensor of a pressureless fluid $T^{\mu\nu} = \rho v^\mu v^\nu$, equation (3) reduces to the generalised geodesic equation (2).

(3) in a gauge covariant theory of gravitation[4], the tensor equations in general relativity are replaced by co-tensor equations. The geodesic equation is replaced by

$$v^\nu v^\mu{}_{*\nu} = 0 \qquad (4)$$

where v^μ is now a co-vector of power -1 and * indicates co-covariant differentiation. It can be shown that equation (4) is a compact form of equation (2).

(4) Dirac[5] has given an action principle from which equation (2) can be derived.

With spherical symmetry, the Newtonian limit of equation (2) gives

$$\frac{d}{dt}(\beta\mathbf{r}) = -\beta\frac{GM_\odot}{r^2}\mathbf{r} \qquad (5)$$

This equation admits the angular momentum integral

$$\beta r^2\dot{\theta} = h = \text{constant} \qquad (6)$$

and therefore the reciprocal radius u satisfies the orbit equation

$$\frac{d^2u}{d\theta^2} + u = \beta^2\frac{GM}{h^2} \qquad (7)$$

Thus, the orbital radius R is given by

$$R \sim \frac{h^2}{GM_\odot\beta^2} \qquad (8)$$

In the gauge covariant theory with multiplicative creation as suggested by Dirac, it can be shown that $\beta \sim t^{-1}$. The result $R \sim t$ is thus confirmed.

There is abundant evidence to favour multiplicative creation over additive creation[3]. The gauge covariant theory is consistent with co-moving multiplicative creation. Roxburgh's suggestion of multiplicative creation at rest does not have a sound theoretical framework and his equation of motion for this case seems to be an *ad hoc* postulate, the consequences of which ought not to be associated with Dirac cosmology.

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[1] Roxburgh, I. W. *Nature*, **261**, 301 (1976).
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ROXBURGH REPLIES—Canuto *et al.*[1] correctly point out that if the equations of motion in the Dirac[2] cosmology are Einstein's equations in Einstein units rather than in atomic units as I assumed[3], then the orbit of the Earth around the Sun increases in time $R_A \propto t$, so that the temperature on the Earth increases only slowly $T \propto t^{1/4}$. This is only the case if matter is created at rest relative to local matter, not if it is created at rest relative to the local cosmological rest frame. In this latter case, even if the equation of motion is Einstein's in Einstein units, we have for a system of $N(\tau)$ particles of mass $m(\tau)$

$$\frac{d}{d\tau}(Nmv) + NMv\frac{1}{N}\frac{dN}{d\tau} = -\frac{GMNm}{r^2{}_E} \qquad (1)$$

and with Nm = constant in Einstein units this gives

$$\frac{d}{d\tau}(Nv) = -\frac{GMN}{r^2{}_E} \qquad (2)$$

and hence $R_E \propto 1/N^2$. In atomic units $R_A \sim tr_E \propto t/N^2 \propto 1/t^3$ so the equilibrium temperature of the Earth satisfies

$$T_E \propto \left(\frac{L}{R_A}\right)^{1/4} \propto \left(\frac{G^7M^5}{R^2{}_A}\right)^{1/4} \propto t^{9/4} \qquad (3)$$

which predicts a temperature of about 180 K when the Earth was formed.

The assumption that Einstein's equations are valid in Einstein units, however, does not uniquely predict that the creation rate $\propto t^2$ or that the scale factor in cosmology is $S(t) \propto t$ in atomic units as in the Dirac model. Indeed, if we demand that there be no cosmological constant in Einstein units then one solution is clearly the Einstein de Sitter Universe with $S(t) \propto t^{2/3}$ in Einstein units. This is satisfied with the creation rate such that $N^\circ(\tau) \propto \tau^n$ for any n (my work, to be published). In atomic units this gives a scale factor

$$S(t) \propto t^{\frac{(3n+1)}{(3(n+1))}}$$

For $n = 0$ this is the classical Dirac cosmology $S \sim t^{1/3}$. The equilibrium temperature on the earth for this model then satisfies

$$T_E \propto t^{\frac{(n-5)}{(4(n+1))}} \qquad (4)$$

and is constant for $n = 5$. The point is that just to demand that Einstein's equations be valid in units in which mN, G, are constant does not determine the model, a whole family of models are possible that satisfy the large number hypothesis and Einstein's equations in suitable units, only if we demand that in atomic units $S(t) \sim t$, or equivalently that in Einstein units we have a static Einstein Universe, as we reproduce the Dirac model.

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Devonian palaeogeography of Northern Britain

DONOVAN *et al.*[1] have recently summarised sedimentological data from the Devonian of North Scotland which they believe invalidate my suggestion of a large-scale sinistral shift along the Great Glen Fault (GGF) in late/post Devonian time[2–4]. Their main arguments are that "the ORS sediments of the Orcadian Basin on both sides of the GGF are very similar in character and show identical history of development" and that palaeocurrent vectors support sedimentation within a single basin. The authors consider the Orcadian Basin to have been of oval shape, the long axis being of the order of 400 km. If a basin of this size can have similar characteristics throughout there is no reason why the outcrops from a basin only about twice as long (corresponding to my proposed 500-km transcurrence) should not exhibit an equally good match of sedimentological and other features. The evolution of the Orcadian Basin may have been strongly linked with lateral and vertical movements of the GGF, causing simultaneous variation in sedimentary facies, tectonic structures and so on over extensive parts of its length. Furthermore, from the palaeocurrent vectors presented (dominantly from west of the fault) one can devise widely different reconstructions within the framework of a single basin: data from the adjoining shelf areas are very necessary before such information becomes relevant to the problem under consideration.

The available data can equally well be fitted to the model of the Orcadian Basin having subsequently become subdivided by a major transcurrent movement. With a 500-km sinistral displacement the East Shetland Basin, which includes thick basal breccias and coarse fluvial conglomerates derived from a metamorphic/plutonic terrain to the west[5], would fit in well with the geology of areas around southern Inverness-shire (west of the GGF).

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Statement

IN September 1976 Dr Robert J. Gullis left our laboratory after having spent two postdoctoral years with us. He had been engaged mainly in measuring the levels of cyclic GMP in neuroblastoma cells and neuroblastoma × glioma hybrid cells. We published four papers on this matter together with him. After Dr Gullis had left, several of my colleagues (M. Brandt, J. Traber and D. van Calker) repeated this work, but were unable to reproduce it. Dr Gullis was therefore asked to return to our laboratory and repeat his essential experiments under supervision. During a 2-week period Dr Gullis carried out four series of experiments. After the experimental incubations, the samples were coded. In none of the experiments was Dr Gullis able to obtain his previous results. Neither morphine nor levorphanol nor the enkephalins nor cholinergic agonists changed the level of cyclic GMP in the hybrid cells.

In some of the publications listed below cyclic AMP was determined in the same samples in which cyclic GMP had been determined. The cyclic AMP assays were carried out by other members of the laboratory. But the printouts from the scintillation counter were left to Dr Gullis for evaluation.

Dr Gullis admitted having invented the results of all his experiments. Thus, I should like to let it be known to the scientific community that the following three publications are based on invented data:

Gullis, R. J., Traber, J. & Hamprecht, B. Morphine elevates levels of cyclic GMP in a neuroblastoma × glioma hybrid cell line. *Nature* **256**, 57–59 (1975).
Gullis, R. J., Traber, J., Fischer, K., Buchen, C. & Hamprecht, B. Effects of cholinergic agents and sodium ions on the levels of guanosine and adenosine 3′ : 5′-cyclic monophosphates in neuroblastoma and neuroblastoma × glioma hybrid cells. *FEBS Lett.* **59**, 74–79 (1975).
Gullis, R. J., Buchen, C., Moroder, L., Wünsch, E. & Hamprecht, B. Opiate-like effects of enkephalin on neuroblastoma × glioma hybrids, in *Opiates and endogenous opioid peptides* (ed. Kosterlitz, H. W.) 143–151 (Elsevier, Amsterdam, 1976).

The data on cyclic AMP were falsified by Dr Gullis in a fourth paper (Figs 3 and 4).
Brandt, M., Gullis, R. J., Fischer, K., Buchen, C., Hamprecht, B., Moroder, L. & Wünsch, E. Enkephalin regulates the levels of cyclic nucleotides in neuroblastoma × glioma hybrid cells. *Nature* **262**, 311–313 (1976).

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DR GULLIS WRITES—I wish to disclose the fact that papers published in several journals with myself as principal author are not reliable. The curves and values published are mere figments of my imagination, and during my short research career I published my hypotheses rather than experimentally determined results. The reason was that I was so convinced of my ideas that I simply put them down on paper; it was not because of the tremendous importance of published papers to the career of a scientist.

Therefore I would like to let it be known that the following papers published while I was working in the laboratory of Dr B. Hamprecht are not reliable.

Gullis, R. J., Traber, J. & Hamprecht, B. *Nature* **256**, 57–59 (1975).
Gullis, R. J., Traber, J., Fischer, K., Buchen, C. & Hamprecht, B. *FEBS Lett.* **59**, 74–79 (1975).
Gullis, R. J., Buchen, C., Moroder, L., Wünsch, E. & Hamprecht, B. Opiate-like effects of enkephalin on neuroblastoma × glioma hybrids, in *Opiates and endogenous opioid peptides* (ed. Kosterlitz, H. W.) 143–151 (Elsevier, Amsterdam, 1976).

Another paper in which I was coauthor and submitted cyclic GMP values is also wrong in terms of the cyclic GMP content (Figs 3 and 4). The paper is

Brandt, M., Gullis, R. J., Fischer, K., Buchen, C., Hamprecht, B., Moroder, L. & Wünsch, E. *Nature* **262**, 311–313 (1976).

I would also like to disclose the fact that the following papers published with Dr C. E. Rowe are purely hypothesis.

Gullis, R. J. & Rowe, C. E. *Biochem. Soc. Trans.* **1**, 849 (1973); *Biochem. J.* **148**, 197–208; 557–565; 567–581 (1975); *J. Neurochem.* **26**, 1217–1230 (1976); *FEBS Lett.* **67**, 256–259 (1976).

This letter is to point out to the scientific community that the results presented in these papers are wrong and based purely on hypothesis. I must take full responsibility for these unfortunate incidents and have consequently suffered. I hope that my experiences are noted by others, and I would like to apologise to the scientific community and the various people involved.

R. J. GULLIS

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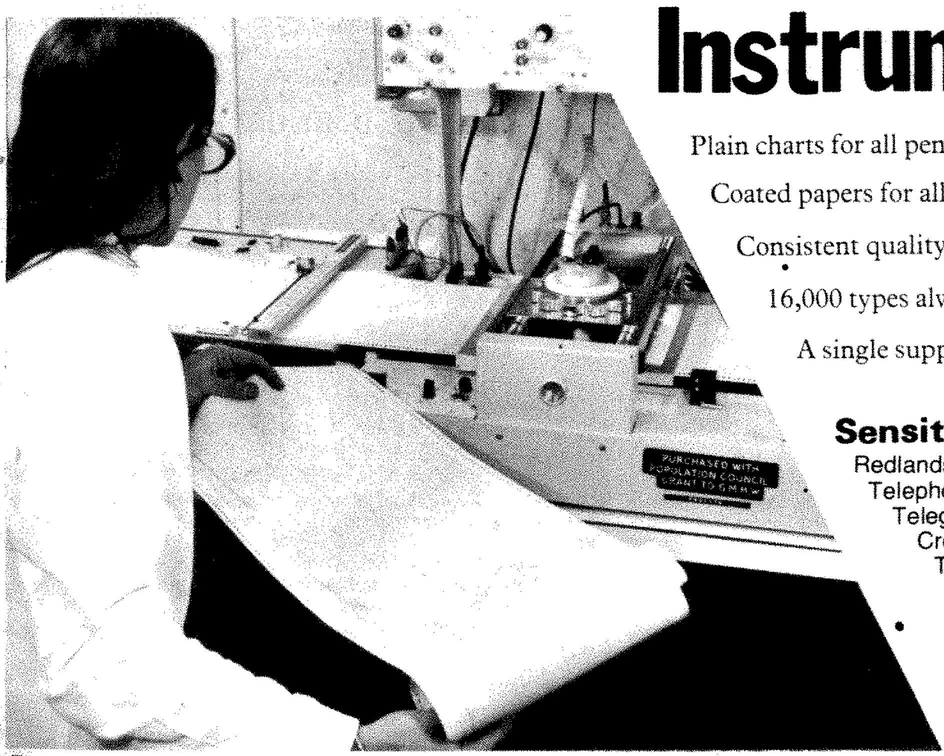

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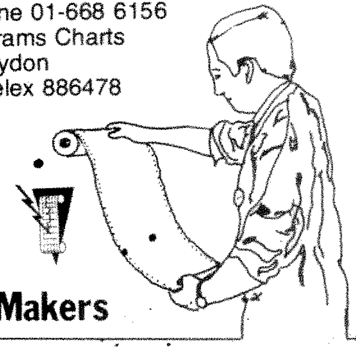

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reviews

Human qualities and political realities

E. H. S. Burhop

Energy and Conflict: The Life and Times of Edward Teller. By S. A. Blumberg and G. Owens. Pp. xvii+492. (Putnam: New York, 1976.) $9.95.

THE role of physics in society has been transformed out of all recognition during the past fifty years. The demonstration that at least some of the discoveries of modern physics have immediate relevance for problems of human society has led inevitably to many aspects of physics research becoming inextricably linked with politics. Those physicists who aspire successfully to lead the work in such fields need not only the conventional gifts that make a great scientist—curiosity, creative imagination, enthusiasm, deep insight, critical scepticism and so on—but also a wide understanding of human and social problems and of political realities. This book describes how one such physicist met the challenge.

Edward Teller is one of a remarkable group of Hungarian-born emigrés (including Eugene Wigner, John von Neumann and Leo Szilard) who made great contributions to the development of nuclear energy in the USA. Teller had shown remarkable aptitude for research in theoretical atomic and nuclear physics and in theoretical chemistry, and his original contributions in these fields are very considerable. Unfortunately he was much less successful in dealing with the political problems to which I have referred, with the result that he has become a controversial figure, forfeiting the respect and friendship of many of the physicists whose good opinion he valued most and rightly or wrongly establishing for himself the image of a rather sinister Professor Strangelove figure to large sections of the public.

This biography of Teller traces his career through his early days as a student and brilliant young research physicist in Budapest, Germany and Copenhagen, his emigration to the USA, his involvement with the early work in neutron physics, and nuclear fission; and, throughout World War II and afterwards, his single-minded commitment to the realisation of a thermonuclear weapon, the so called superbomb. His campaign to secure agreement to a crash program to develop the hydrogen bomb brought him into conflict with many of his scientific-colleagues—especially Robert Oppenheimer, who had been in charge of the development of the wartime fission bomb at Los Alamos. Teller was given the green light to go ahead. In the cold war atmosphere of the time that produced the hysteria of McCarthyism, of which most of Teller's colleagues were thoroughly ashamed, Oppenheimer's opposition to the development of the hydrogen bomb was represented as something sinister, and Eisenhower ordered that Oppenheimer's security clearance should be subject to a hearing, as required by law, to show cause why it should not be rescinded. In the subsequent hearings before a specially constituted commission, Teller's evidence was decisive. The counsel for the Commission cross-examining Teller, put to him the key question "Do you or do you not believe that Dr Oppenheimer is a security risk?" Teller's reply included the damning words "I would like to see the vital interests of this country in hands which I understand better and therefore trust more".

This moment was a watershed in Teller's career. He has never been able to live it down. The Commission went on to remove Oppenheimer's clearance. Oppenheimer continued with his scientific work as Director of the Institute of Advanced Studies at Princeton. But he was a sensitive man and he never fully recovered from the ordeal through which he had had to pass.

Teller was given the opportunity to build up a new weapons laboratory at Livermore, California, in competition with the Los Alamos Laboratory, set up by Oppenheimer during the war, and was able to realise his ambition, which had become almost an obsession, to provide the US Army with its hydrogen bomb.

The story of the development of the hydrogen bomb is a fascinating one and both it, and the monumental clash between the personalities of Oppenheimer and Teller is well told. Both were equals as physicists but it would be hard to imagine two more different personalities. Oppenheimer was a man of great culture with very broad interests and understanding of social problems and their perspectives. Before the War he had identified the true nature of fascism and realised that only by building a popular front with all forces opposed to it, including the communists, could it be fought and defeated.

Teller was much more limited in his understanding of the broader problems of society. His attitudes were no doubt conditioned by his background and education in Hungary and Germany as the son of a prosperous Jewish lawyer. His family suffered discrimination under the Bela Kun regime of 1919 on account of their bourgeois origins. They witnessed the rise of anti-Semitism in Hungary during the period of the Horthy regime, eventually culminating in the unspeakable excesses against the Jews of the Nazi Arrow Cross Movement. He emerged with a simplistic, uncritical equation of Communism with Nazism and an irrational hatred of the Soviet Union which has undoubtedly coloured his judgment. While Oppenheimer looked beyond the era of the cold war with its "peace by mutual terror" to an era of negotiation, détente and "peace through understanding" which would make the qualitative escalation of nuclear weapons unnecessary, Teller apparently saw and still sees only the need for weapons escalation unlimited.

This book has no doubt been originally conceived as an apologia for Teller, to try to do something to restore his image. Nevertheless it is by no means an uncritical eulogy, and brings out some of the weaknesses as well as the favourable qualities of its subject. It seems to deal objectively with the clash of personalities between Oppenheimer and Teller, the highpoint of the narrative. Teller emerges from the book as a warm and friendly man and, to his credit, with a sincere detestation of secrecy in science. Yet, in the opinion of the reviewer, such desirable personal qualities cannot balance out the great harm his uncompromising hawkish attitudes have done to the prospects of building a peaceful and secure future for mankind. He represents an example and a warning of a very talented scientist with many laudable and human qualities, whose lack of understanding of the social and political forces at work in our society has been a misfortune for himself and his colleagues. ☐

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Reproduction of eukaryotic cells

Reproduction of Eukaryotic Cells. By D. M. Prescott. Pp. ix+177. (Academic: New York and London, October, 1976.) $14.50;. £8.85.

ACCORDING to the blurb this book "integrates recent discoveries about the molecular biology of the cell cycle into a comprehensive explanation of cell reproduction". In his preface the author wisely states his purpose more modestly ". . . to organise into a single source the principal facts and observations on the cell life cycle . . . in an effort to increase our overall understanding of how these cells reproduce themselves and how this reproduction is regulated". The book interprets eukaryote cells to be mainly vertebrate derived, but we do get occasional forays into yeasts and amoeba. The monograph is intended to be (and indeed is) complementary to Mitchison's book, *Biology of the Cell Cycle* (Cambridge University: Cambridge and London, 1971), which dealt extensively with microorganisms.

After briefly introducing the cell cycle and discussing the pattern of cell mass increase between successive divisions the author plunges straight into cell synchronisation techniques. For many workers, cell synchrony is a prerequisite for any successful biochemical or molecular biological study of cell cycle control. The author does not make the usual mistake of ignoring the variability of G_1 but he seems puzzled by the failure of many attempts to achieve perfect cell synchrony. In spite of the 560 references (mostly we are told published since 1971), no mention is made of models of the cell cycle which not only predict that G_1 must of necessity be variable, but put this variation on a quantitative footing (Burns and Tannock, *Cell Tissue Kinet.*, **3**, 321, 1970; Smith and Martin, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1263, 1973). Whether these explanations of the variation of G_1 are accepted or not the variability of G_1 is an undeniable and unavoidable fact. As a consequence, temporal studies on the biochemistry of cell division are fraught with pitfalls. For instance, even within a mitotically synchronised population some cells enter S-phase within a few hours but other cells do not start DNA synthesis until many hours later. As a result, biochemical phenomena observed in an "early G_1" population are a mixture of events occurring in some cells just before DNA synthesis begins and events that occur in cells that will sit around for many hours before initiating S-phase. In "mid G_1" or "late G_1" the situation is worse. This unavoidable problem means that much of the biochemistry of G_1 is at best of questionable value and at worst useless. Synchronisation at other stages of the cell cycle is slightly more successful, but it is G_1 that is at the centre of cell proliferation control. A knowledge of what happens in G_1 will only come with a proper understanding of its variable nature and the nature of its variation. Until then even the best of monographs can throw little light on the subject.

Although those who have not been exposed to the cell cycle literature will find much of this book of interest, it is a pity that the author has drawn his brief so narrowly. The "molecular biology" we are promised on the dust-jacket turns out to be a rather dry series of studies with inhibitions of protein and RNA synthesis. There is nothing (for instance) on the role of microtubules in the formation of the mitotic spindle, little on the process of cytokinesis and very little (apart from a discussion of RNA and protein synthesis) about the process of mitosis itself. There is a chapter on that slumping commodity cyclic AMP but little indication of what interesting futures the author would invest in.

In short, although the book is a useful source of references about the bare essentials of the cell cycle it fails to convey much of the excitement which surrounds the study of cell division and its control. **Robert Shields**

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Characteristic seeds

The Seeds of Dicotyledons. By E. J. H. Corner. Vol. 1: pp. ix+311; Vol. 2: pp. 552 (illustrations.) (Cambridge University; Cambridge, London and New York, 1976.) Vol. 1: £15; Vol. 2: £25.

THE flowering-plant taxonomist, struggling with a welter of data, may at times be forgiven for regarding Occam's razor as a more useful tool than the microtome. Not so his brother the phylogenist, for Nature has concealed the origins and ancestry of the angiosperms with such skill, that every scrap of evidence may be required to unravel the mystery, and transform hypothesis into proven fact. In this respect, seeds (surely of some significance in seed-plants) have been seriously underrated by taxonomists and phylogenists alike, perhaps because they are not the easiest things in the world to examine, or to describe.

Professor Corner claims that every natural family of dicotyledons has a characteristic seed, and that, in consequence, the classification of families into orders should be consistent with their seeds. To substantiate this claim 218 pages of volume 1 of *The Seeds of Dicotyledons* consist of brief (but perfectly ample) descriptions of seeds and ancillary structures from most dicotyledonous families, together with descriptions of the seeds of many genera within these families. Volume 2 furnishes the necessary illustrations, 647 plates in all, comprising what must amount to several thousand individual figures, each a clear *camera lucida* outline, the salient features emphasised by judicious stippling and shading. The amount of work involved in sectioning (free-hand) so much material at every stage of development from unfertilised ovule to ripe seed, and in illustrating the resultant observations, must have been stupendous, so that it is almost mean to suggest that the plates might have been even more useful had the individual figures and corresponding legends been more uniformly labelled with numbers or letters.

The phylogenetic conclusions which Professor Corner reaches as a result of his seed studies are (coming from the father of Durianology) not quite so startling as might have been supposed. All depends upon one's criteria of primitiveness. Professor Corner regards the elaborations of the Myristicaceous seed as the closest approximation to the primitive; thereafter it is but a step to the *Magnoliaceae, Annonaceae, Lauraceae* and *Monimiaceae*, and indeed to a situation which, through Hutchinson, Takhtajan and others, has become so familiar that we may well be too inclined to take it for granted. If so, the hypothesis that *Paeoniaceae, Cucurbitaceae* and *Convolvulaceae* belong to the same phyletic line; that *Grossulariaceae* should be allied to *Cruciferae* and *Polygalaceae*, and *Proteaceae* to *Papaveraceae* and *Fumariaceae* will jolt us out of complacency, and perhaps make us question (as Professor Corner anticipates) if "agreement in seed-structure should be employed to align such diverse plants in a phyletic series". I take his word for it that "it can stand enquiry". It is nonetheless surprising. **R. D. Meikle**

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